Toxicological Profile for 2,4-Dichlorophenoxyacetic Acid (2,4-D)

July 2020

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

2,4-Dichlorophenoxyacetic acid (2,4-D) is a free acid pesticide widely used in the United States. While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D. Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester. Almost 90–95% of total global use is accounted for by dimethyl amine salt and ethylhexyl ester. 2,4-D and its different chemical forms are listed as an ingredient, either as the singular active ingredient or in conjunction with other ingredients, in about 600 agricultural and residential products. 2,4-D is one of the most widely used agricultural herbicides in the United States with approximately 39 million pounds applied to crops in 2013; pasture and hay fields and wheat, soybean, and corn crops receive the greatest applications. It is also applied to residential or commercial turf for the elimination of a wide variety of broadleaf weeds without causing harm to the grass.

The dominant process affecting the overall environmental fate, transport, and bioaccumulation of 2,4-D is degradation by microbiological activity. 2,4-D has been shown to undergo degradation in pure cultures by particular species of microorganisms. The two main pathways of degradation are via a hydroxy-phenoxy acetic acid intermediate or by the corresponding phenol. The half-life of 2,4-D was about 6 days when it was applied to a mineral soil maintained under aerobic conditions. 2,4-D is likely to migrate through the soil and into groundwater since it has high mobility in soils under varying conditions. 2,4-D is not expected to volatilize from water or soil surfaces since most forms of 2,4-D are supplied as amine salts, which do not volatilize, and the ester forms are rapidly transformed to the corresponding acid, which will exist as an anion under environmental conditions. Data suggest that bioconcentration of 2,4-D does not occur to a significant extent in aquatic organisms.

The general population may be exposed to 2,4-D during and after its use in residential and recreational areas. 2,4-D applications often occur to residential lawns, golf courses, parks, cemeteries, and other grassy areas. Since 2,4-D is also used on aquatic weeds, swimmers may also be exposed. 2,4-D can unintentionally be transported into residences if clothing or shoes containing this substance are worn indoors or if pets track in 2,4-D from recently treated lawns. The general population can be exposed to 2,4-D by ingesting food or water contaminated with it or through dermal contact with it when used in residential settings (lawn applications). Populations living within or very near areas of heavy agricultural

2,4-D use have an increased risk of exposure to relatively larger amounts of 2,4-D through dermal contact with contaminated plants, soils, or surface waters or by inhalation of the mist formed from the applied herbicide. Those likely to receive the highest exposures are those who are involved in the production, formulation, handling, and/or application of 2,4-D. Dermal contact appears to be the major route of exposure for workers, although inhalation exposure and accidental ingestion via hand-to-mouth activity is possible.

Children are expected to be exposed to 2,4-D by the same routes that affect adults. Small children are more likely to come into contact with 2,4-D residues that may be present in soil and dust, due to increased hand-to-mouth activity and playing habits. However, dermal contact with house dust contaminated with small residues of 2,4-D is most likely the major route of exposure for children. Treated play areas (lawns) and pets that may have come in contact with 2,4-D on treated lawns is another possible source of exposure. No human data were located regarding 2,4-D in breast milk; therefore, an adequate determination of the importance of this route of child exposure has not been made.

1.2 SUMMARY OF HEALTH EFFECTS

Information regarding health effects in humans following exposure to 2,4-D comes from case reports of accidental or intentional ingestion of herbicide formulations containing 2,4-D, accidental inhalation and/or skin contact with 2,4-D in products used by farmers and professional residential applicators and homeowners, and occupational exposure during manufacture. Effects that have been reported following oral or dermal exposure to high amounts of 2,4-D include tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects characterized by sensory and motor abnormalities. In two reports of dermal exposure, signs and symptoms of peripheral neuropathy persisted for a long time. Some of these studies estimated exposure levels and/or measured levels of 2,4-D in the body. A report estimated an ingested dose of approximately 80 mg/kg in a fatal case. In another fatal case, the investigators estimated that the subject had ingested at least 25–35 g of 2,4-D (357–500 mg/kg for a 70 kg body weight). However, there is a report of two individuals who survived after ingesting approximately 40 and 140 g of 2,4-D (571 and 2,000 mg/kg) in herbicide products. These numbers are the result of the combined action of 2,4-D and other substances in commercial formulations. In addition, whether or not deaths occurred may be related to the time elapsed between poisoning and beginning of emergency medical treatment.

1. RELEVANCE TO PUBLIC HEALTH

Numerous epidemiological studies, mostly case-control and cohort studies, have examined potential associations between exposure to 2,4-D and multiple health outcomes including respiratory effects, endocrine effects, ocular effects, body weight effects, immunological effects, neurological effects, reproductive effects, developmental effects, various cancers, and death.

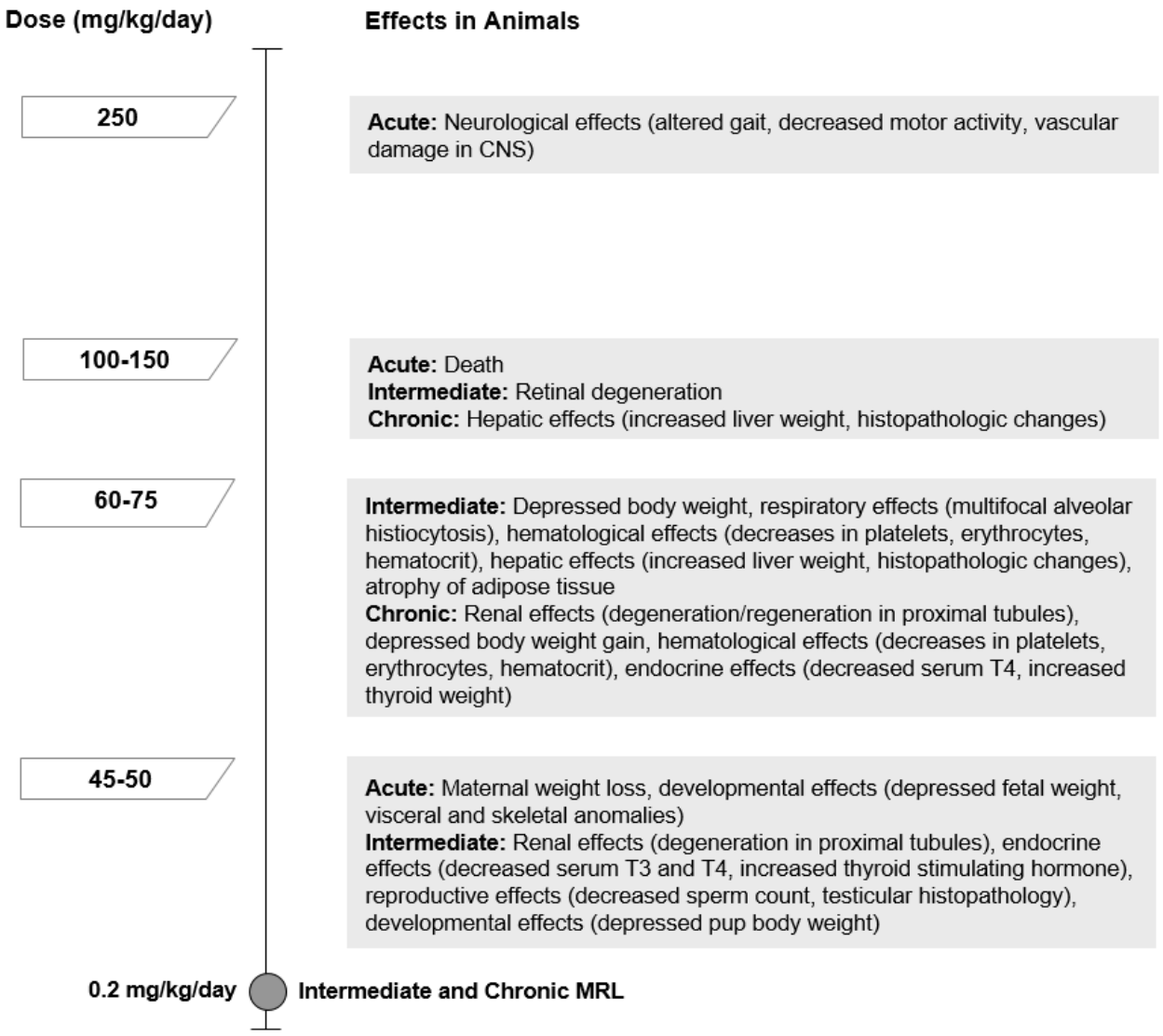
While some of the human studies reported associations between use/exposure to 2,4-D and adverse health outcomes, other studies did not. Pesticide applicators and farm workers are likely to be exposed to multiple chemicals, and even if analyses can be conducted for exposures to individual chemicals, a significant association between use/exposure and increased prevalence of an adverse health outcome does not necessarily imply causality. In general, limitations to the interpretation of reported associations include lack of relationship with frequency or amount of 2,4-D usage, duration of exposure, and/or limited numbers of cases.

The database in animals is extensive and consists almost exclusively of studies that employed the oral route of exposure. Systemic effects reported in repeated exposure oral studies in animals include hematological alterations in rats (decreased hematocrit, platelets, and erythrocyte counts); hepatic effects in rats (histological alterations); renal effects in rats and mice; alterations in thyroid hormone levels in rats; and ocular effects in rats. Review of available animal studies resulted in the conclusion that the kidney is the most sensitive target of 2,4-D toxicity in laboratory animals; 2,4-D kidney toxicity is a presumed health effect for humans.

As illustrated in Figure 1-1, the most sensitive noncancer effects of repeated-dose oral exposure to 2,4-D are kidney effects and developmental effects. Depressed body weight, hematological effects, hepatic effects, endocrine effects, and ocular effects occur at higher exposure levels.

Body Weight Effects. Fofana et al. (2000) reported 3–6% maternal body weight loss among rats administered 2,4-D by gavage at 50–110 mg/kg/day during gestation days (GDs) 6–15; however, the study report only noted that there was no maternal body weight loss among controls (i.e., data regarding maternal body weight gain during the same period were not presented). Bortolozzi et al. (1999) reported 11–12% depressed body weight in rat pups receiving 2,4-D from the food at 70 mg/kg/day during postpartum days 23–75 or 90 after 28 days of maternal exposure. As much as 54% depressed maternal body weight gain was noted for rat dams gavaged with 2,4-D at 100 mg/kg/day during GDs 1–19 (Mazhar et al. 2014). Up to 20% depressed body weight gain was observed in female rats receiving 2,4-D from the diet for 2 years at 75 mg/kg/day (Charles et al. 1996a; EPA 1996a).

Figure 1-1. Health Effects Found in Animals Following Oral Exposure to 2,4-D



Hematological Effects. Repeated oral exposure of rats at doses in the range of 75–100 mg/kg/day resulted in hematological effects including decreases in platelets, hematocrit, and erythrocyte counts in some studies (Charles et al. 1996a, 1996b; EPA 1996a); however, other studies found no 2,4-D treatment-related hematological effects at doses as high as 90–300 mg/kg/day (EPA 1984; Gorzinski et al. 1987).

Renal Effects. Results from a variety of animal studies identify the kidney as a common target of 2,4-D toxicity. However, there is a degree of uncertainty regarding lowest-observed-adverse-effect levels (LOAELs) for adverse kidney effects. One 13-week study reported a LOAEL of approximately 7.1 mg/kg/day for histological lesions in the kidneys of rats. However, other rat studies of ≤13 weeks

reported LOAELs for histological alterations in the kidneys only at doses ≥20 mg/kg/day. The toxicological significance of the results from some studies is not clear (e.g., alterations in the kidneys from rats and mice characterized as increased homogeneity of the cytoplasm and decreased vacuolization of cells in the renal cortex).

Ocular Effects. Intermediate- and chronic-duration oral studies reported ocular effect such as cataracts and retinal degeneration at doses in the range of 150–300 mg/kg/day (Charles et al. 1996a, 1996b; EPA 1996a).

Endocrine Effects. Decreases in serum thyroid hormones were reported in some intermediate- and chronic-duration studies of laboratory animals receiving 2,4-D from the food at doses in the range of 50–150 mg/kg/day (Charles et al. 1996a, 1996b; EPA 1996a, 1996b; Marty et al. 2013).

Developmental Effects. Developmental effects following gestational exposure of rats and/or mice to 2,4-D at maternal doses in the range of 50–150 mg/kg/day include depressed fetal and/or postpartum pup weight, increased incidence of soft tissue and skeletal anomalies, increased resorptions, and increased pup mortality (Chernoff et al. 1990; Fofana et al. 2000, 2002; Kavlock et al. 1987; Mazhar et al. 2014; Schwetz et al. 1971). Gestational and postpartum exposures of rats at maternal doses in the range of 50–126 mg/kg/day and continued dosing of pups directly resulted in effects including depressed pup weight, neurobehavioral alterations, decreased pup viability, developmental effects on the prostate and liver, and alterations in bone histopathology (Bortolozzi et al. 1999; EPA 1986, 1987b; Hansen et al. 1971; Marty et al. 2013; Pochettino et al. 2016; Saghir et al. 2013a, 2013b; Troudi et al. 2012a, 2012b).

Cancer Effects. 2,4-D has been evaluated for possible associations with a variety of cancer types (lymphatic system cancers, gastrointestinal cancer, breast cancer, cancers of the nervous system, prostate cancer, and others) (e.g., Goodman et al. 2015; Flower et al. 2004; Hoar et al. 1986; Pahwa et al. 2006; Smith et al. 2017; see Section 2.19 for full list of citations). Cancer of the lymphatic system, particularly non-Hodgkin's lymphoma (NHL), has received the most attention. Some case-control studies reported that exposure to 2,4-D increased the risk of NHL, but others did not. The case-control studies included agriculture exposure, residential use of 2,4-D, exposure during manufacture, or children from parents participating in the Agricultural Health Study (AHS). Studies that examined cause-specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-D and related salts did not find patterns suggestive of a causal association between exposure to 2,4-D and any particular cause of death, including NHL.

The carcinogenicity of 2,4-D has been evaluated in a number of animal cancer bioassays; species evaluated include rats, mice, and dogs (Charles et al. 1996a; EPA 1996a, 1996b, 1987a; Hansen et al. 1971). These animal cancer bioassays did not provide convincing evidence of 2,4-D carcinogenicity.

The U.S. Environmental Protection Agency (EPA 2005a) has assigned 2,4-D to carcinogenicity Group D, "not classifiable as to human carcinogenicity." The International Agency for Research on Cancer (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). In discussing potential mechanisms by which 2,4-D could induce cancer, IARC noted that the evidence that 2,4-D induces oxidative stress that can operate in humans is strong, the evidence that 2,4-D is genotoxic is weak, the evidence that 2,4-D causes immunosuppression is moderate, the evidence that 2,4-D modulates receptor activity is weak, and the evidence that 2,4-D alters cell proliferation or death is weak. Recently, Canada's Pest Management Regulatory Agency (PMRA 2016) concluded that 2,4-D cannot be classified as a human carcinogen based on the inconsistent epidemiological associations, the recognition that that there are many other factors that may contribute to the etiology of the reported cancer cases, information from the PMRA's incident report database, and the fact that the weight of evidence from animal studies designed to show causality did not support a carcinogenic effect.

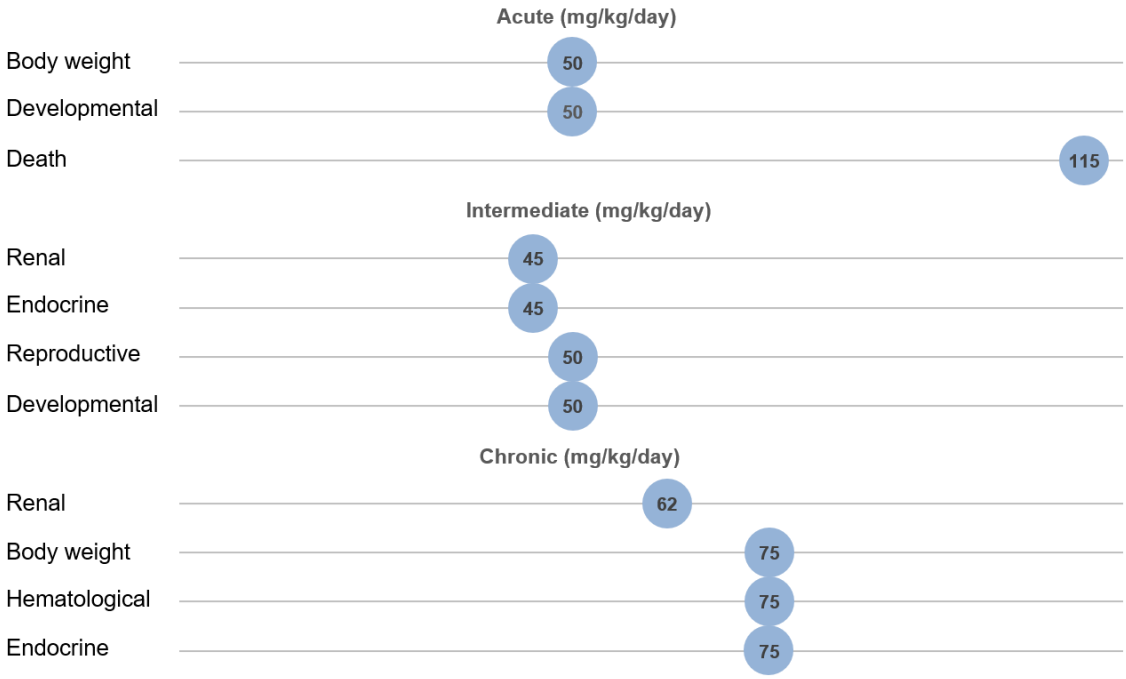
1.3 MINIMAL RISK LEVELS (MRLs)

As discussed in Appendix A, the inhalation database was not considered adequate for derivation of acute-, intermediate-, or chronic-duration inhalation MRLs.

As shown in Figure 1-2, the kidney is the most sensitive target of 2,4-D toxicity following oral exposure. The oral database was not considered adequate for derivation of an acute-duration oral MRL for 2,4-D. The oral database was considered adequate for derivation of intermediate- and chronic-duration oral MRLs for 2,4-D.

Figure 1-2. Summary of Sensitive Targets of 2,4-D – Oral

The kidney is the most sensitive target of 2,4-D oral exposure.
Numbers in circles are the lowest LOAELs for all health effects in animals.
No reliable dose response data were available for humans.



The MRL values for 2,4-D are summarized in Table 1-1 and discussed in greater detail in Appendix A.

Table 1-1. Minimal Risk Levels (MRLs) for 2,4-D[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure (ppm)					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	Insufficient data for MRL derivation				
Intermediate	0.2	Kidney lesions	16.6 (NOAEL)	100	Marty et al. 2013
Chronic	0.2	Kidney lesions	16.6 ($BMDL_{10}$)	100	Charles et al. 1996a; EPA 1996b

[a]See Appendix A for additional information.

2,4-D = 2,4-dichlorophenoxyacetic acid; BMDL = 95% lower confidence limit on the benchmark dose (subscripts denote benchmark response: i.e., 10 = exposure concentration associated with 10% extra risk); NOAEL = no-observed-adverse-effect level

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.

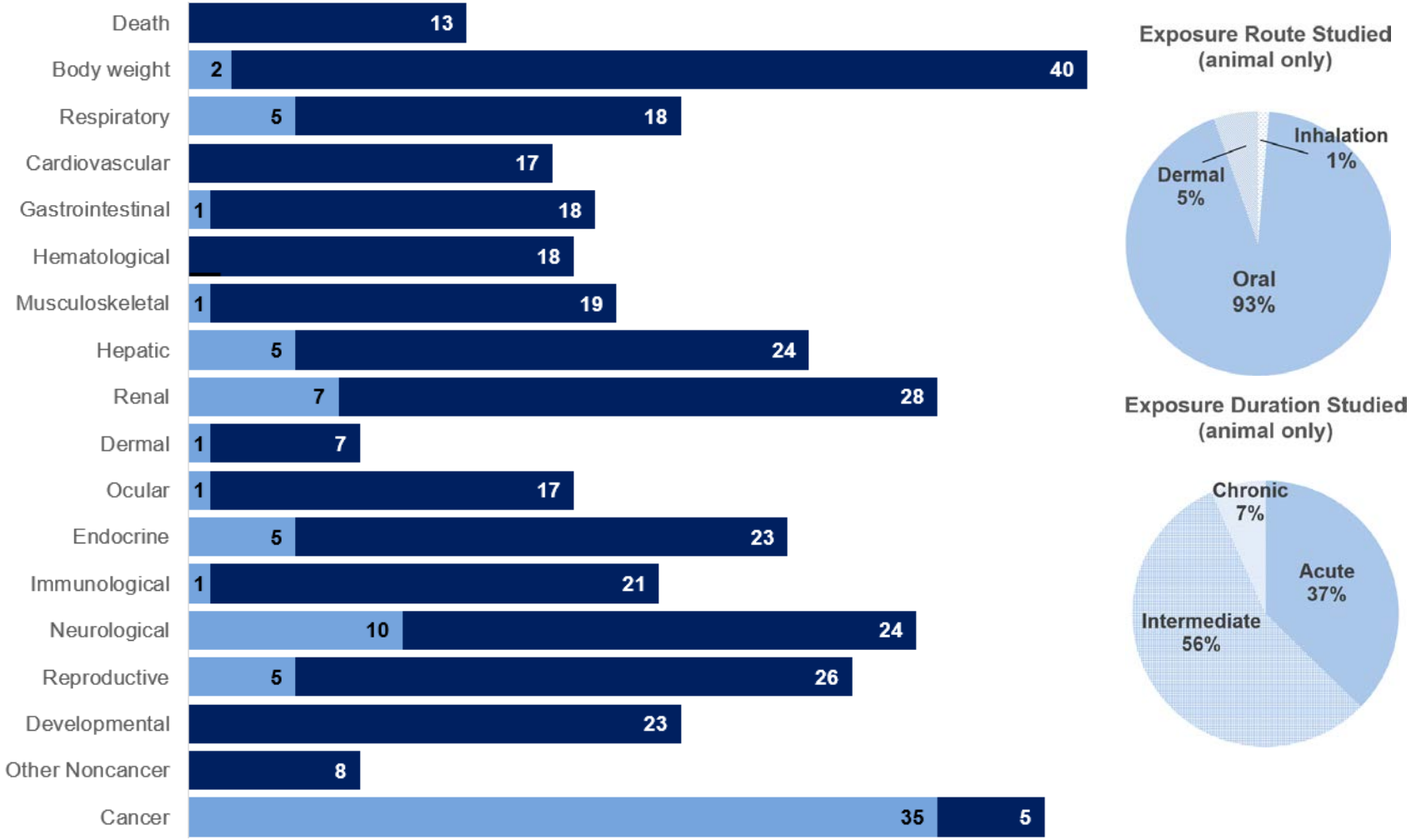
- **Renal endpoints:** Available human data are restricted to a few case reports of kidney damage following intentional ingestion of 2,4-D products. Kidney damage has been reported in a variety of animal studies that employed oral exposure to 2,4-D.

- **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)

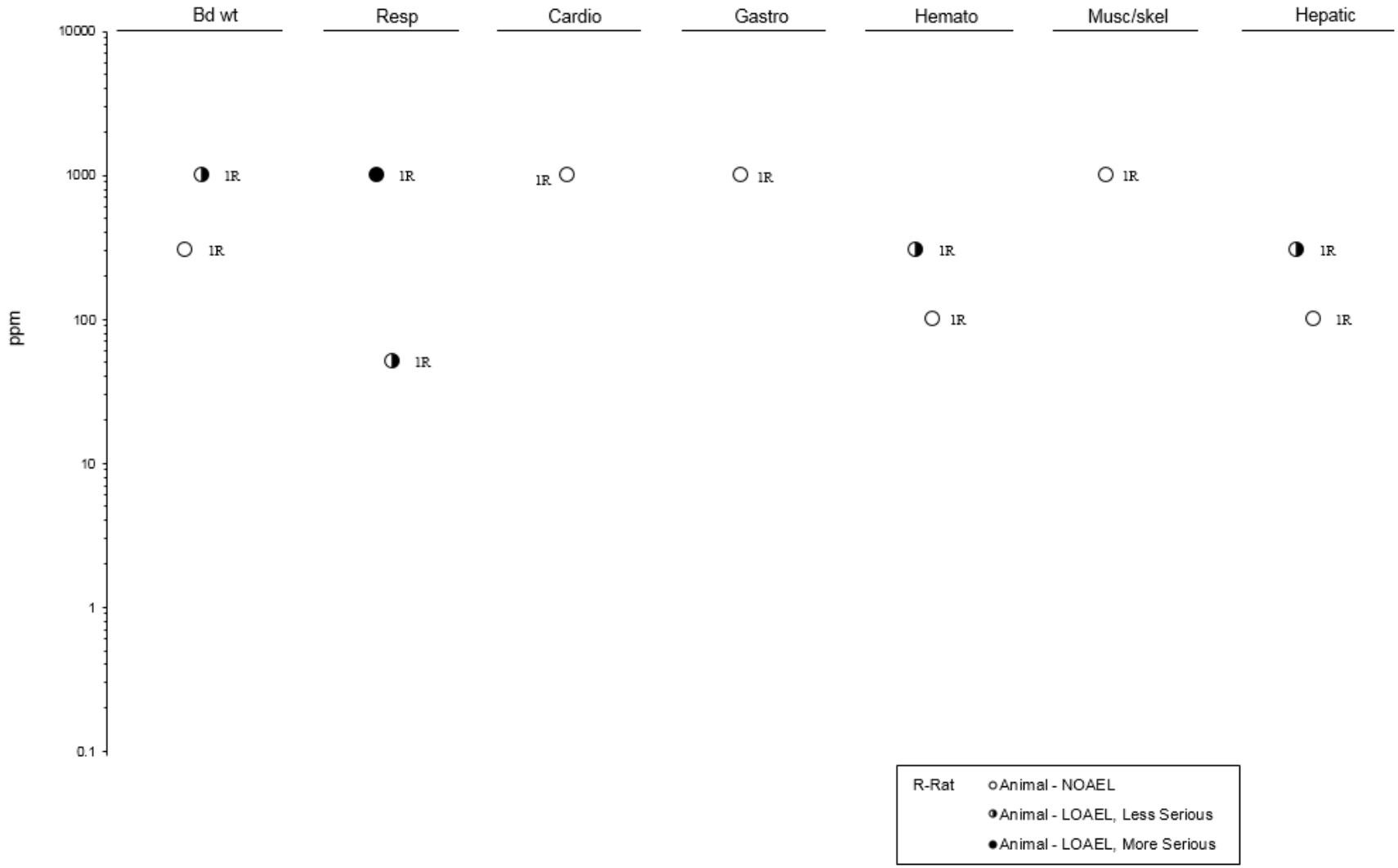


Figure 2-2. Levels of Significant Exposure to 2,4-D – Inhalation

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)

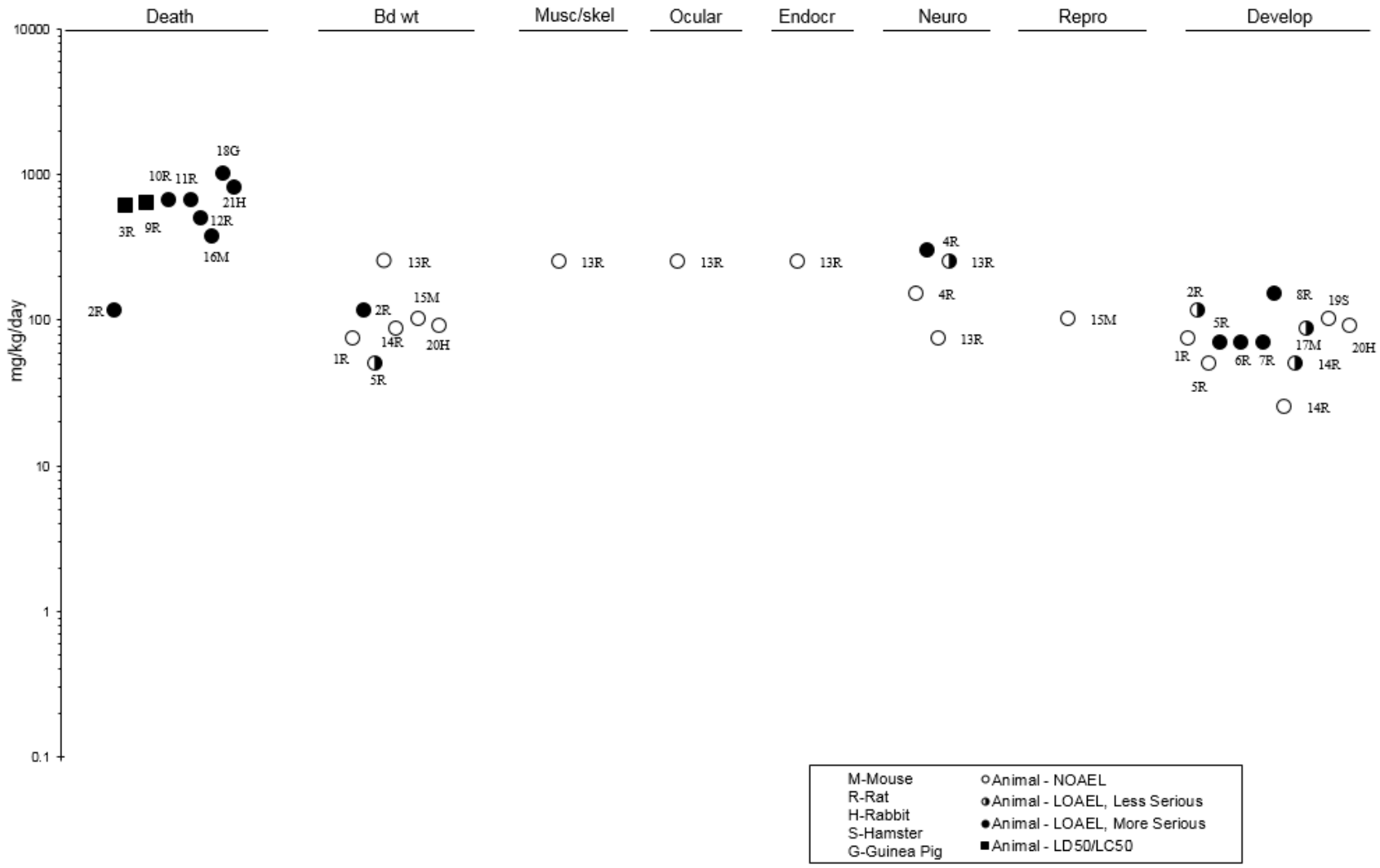


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

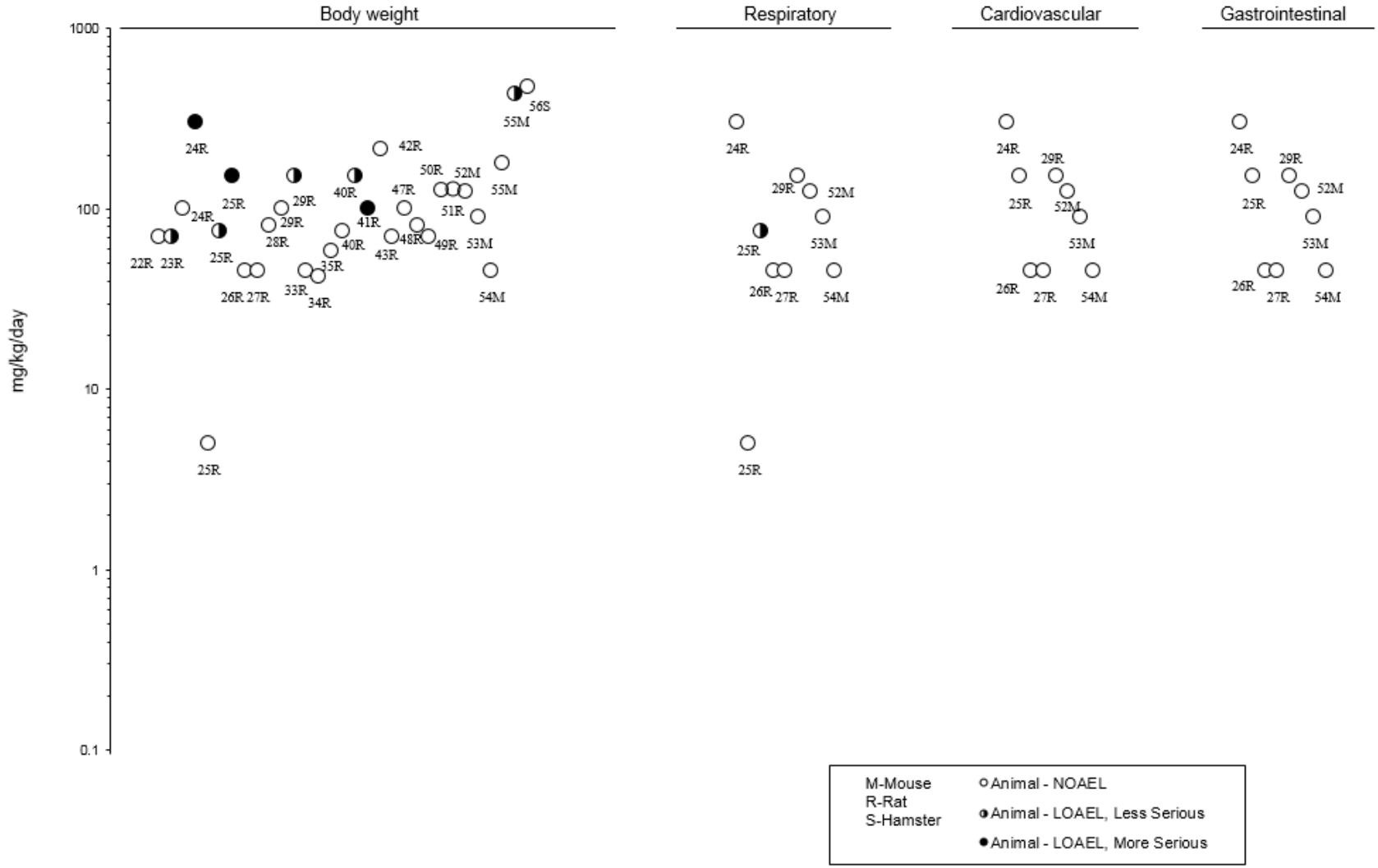


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

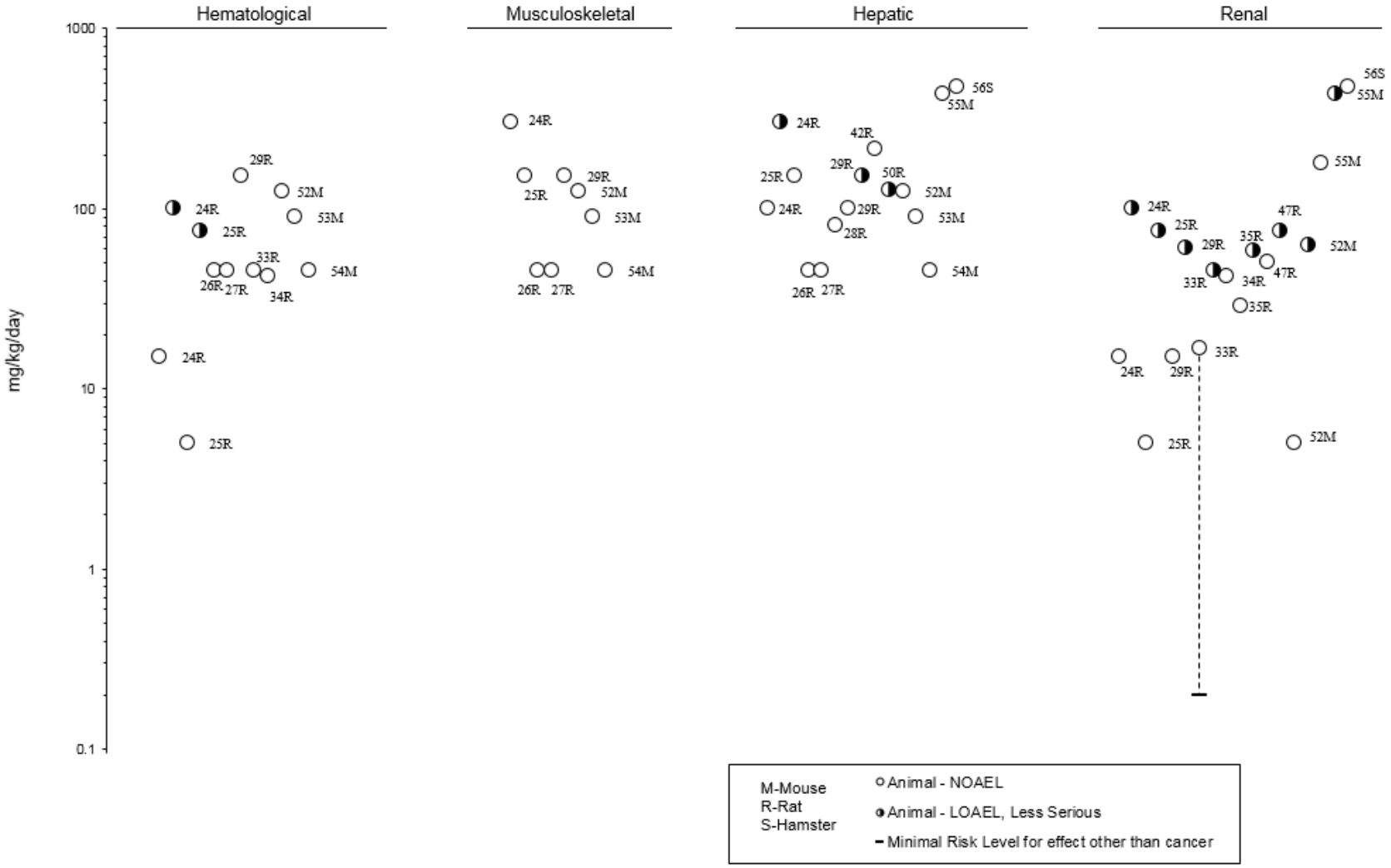


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

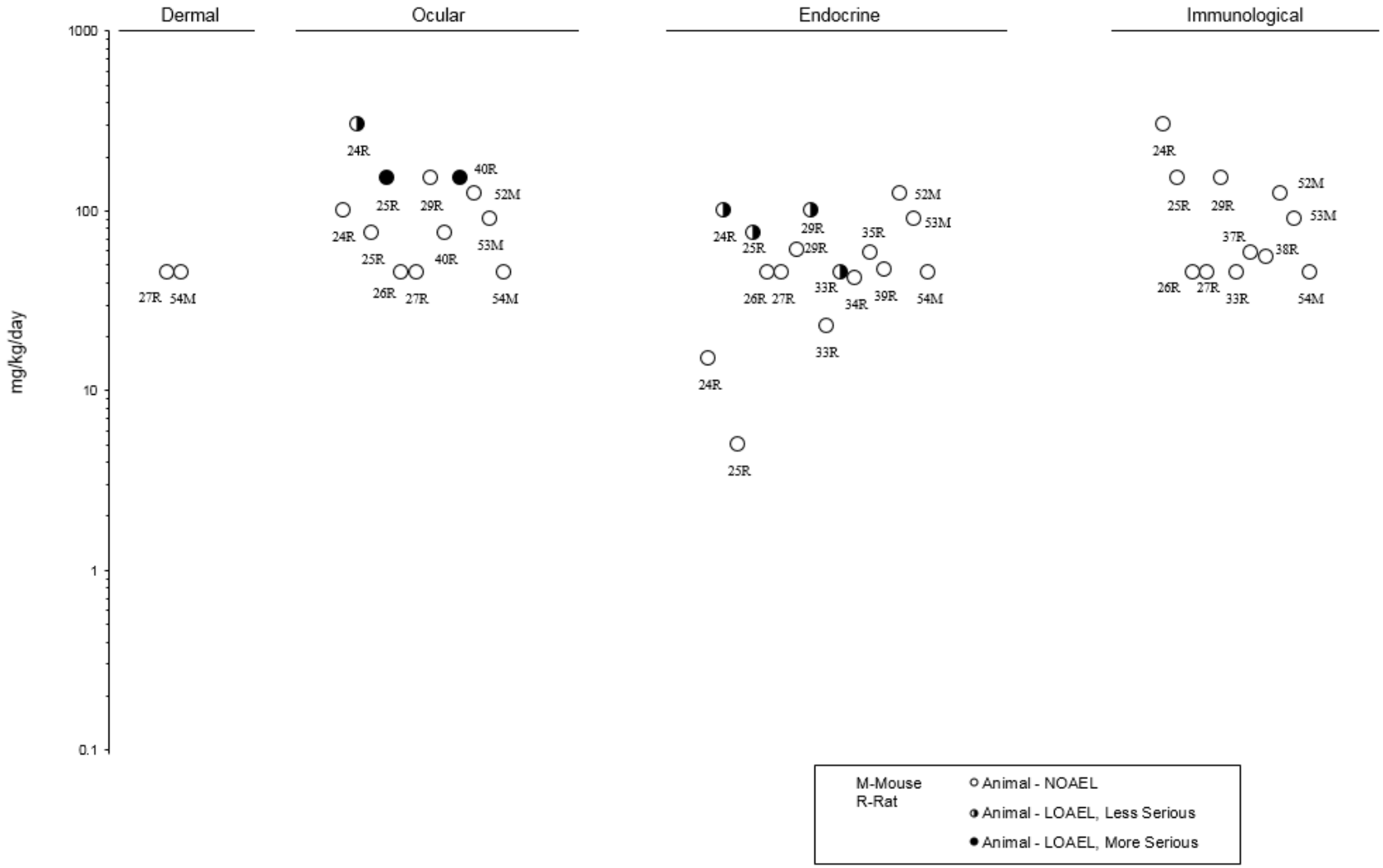


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Intermediate (15-364 days)

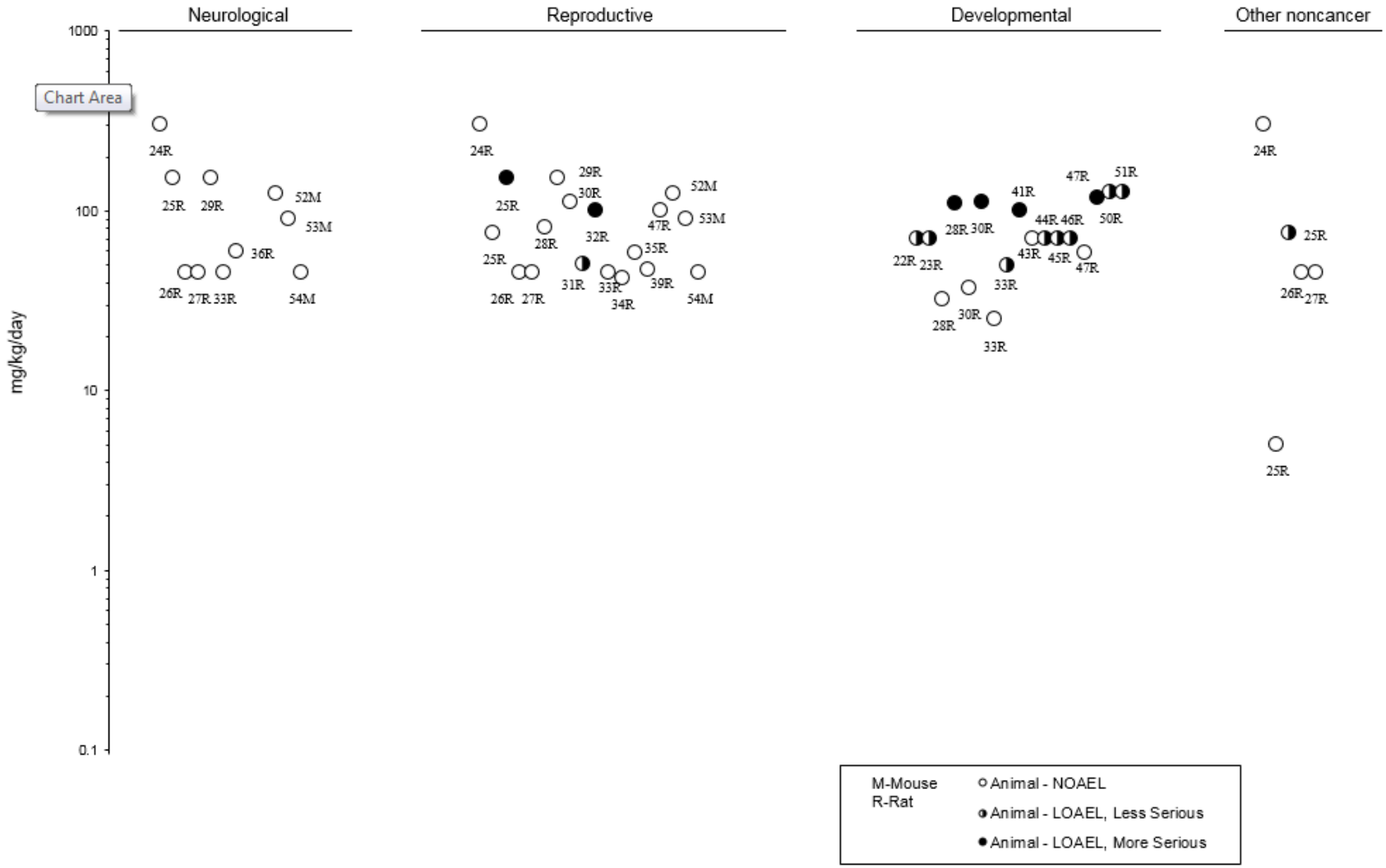


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Chronic (≥365 days)

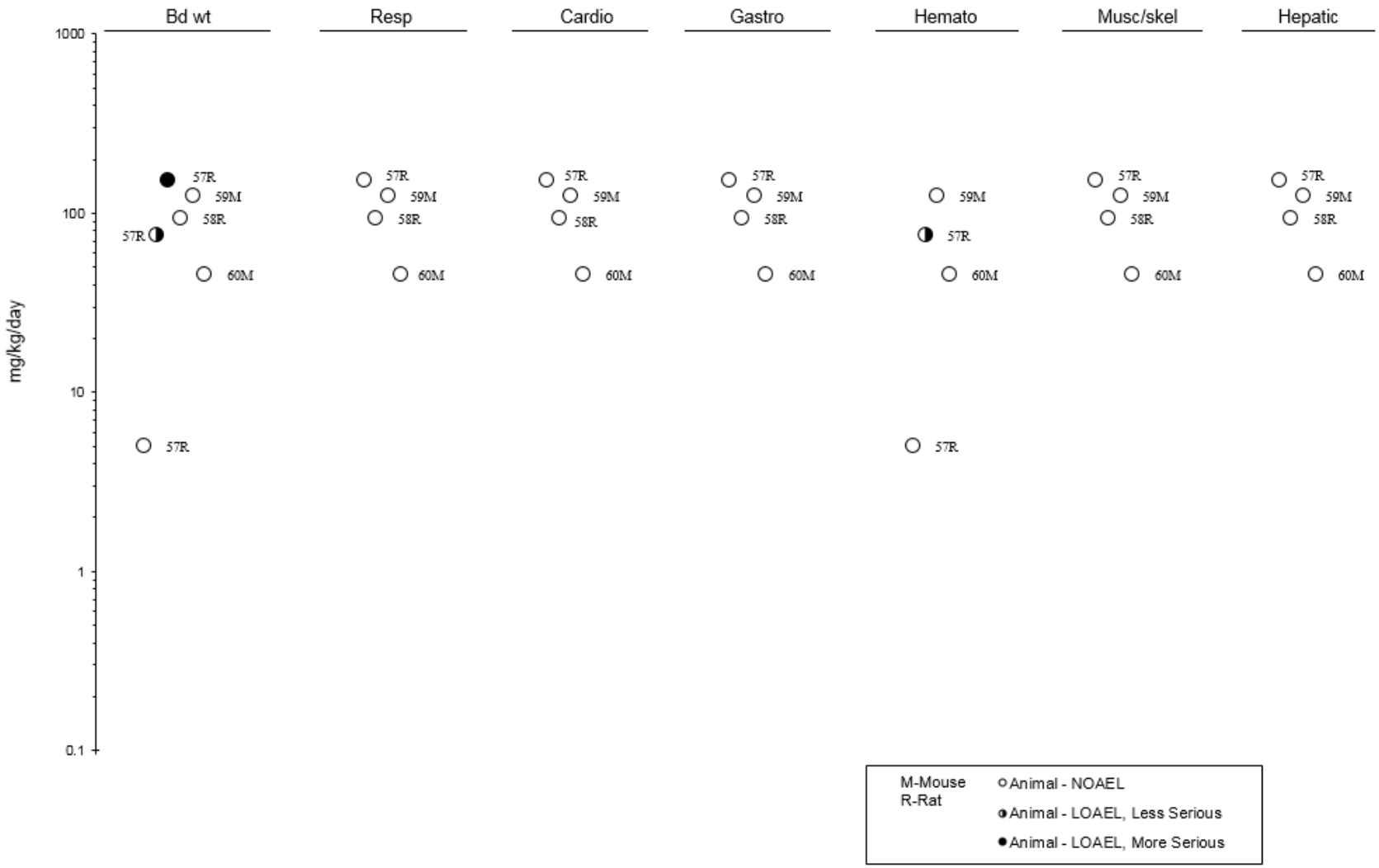


Figure 2-3. Levels of Significant Exposure to 2,4-D – Oral

Chronic (≥365 days)

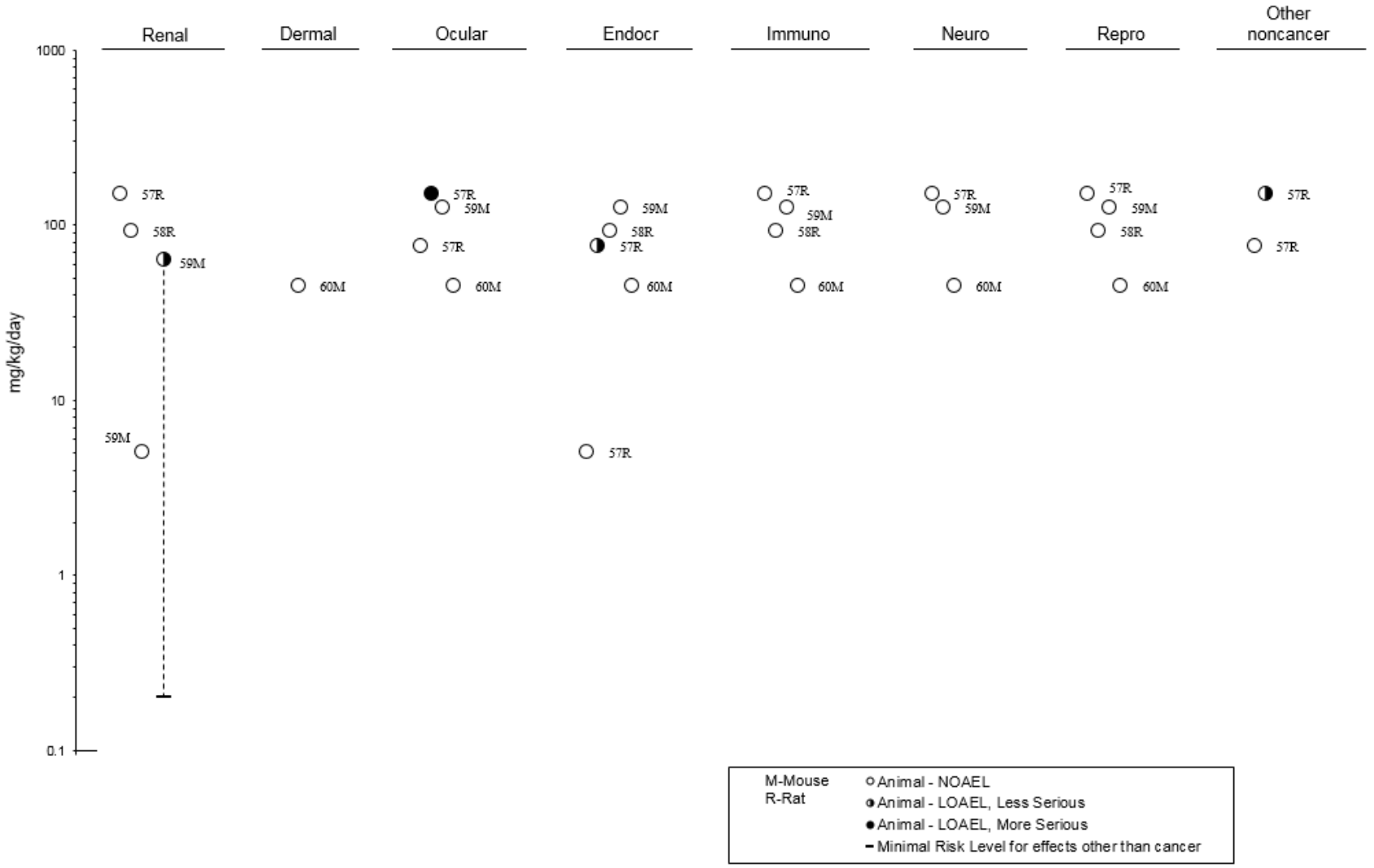


Table 2-3. Levels of Significant Exposure to 2,4-D – Dermal

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).

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

3.1 TOXICOKINETICS

- 2,4-D is readily absorbed from the gastrointestinal tract and is expected to be readily absorbed from the respiratory tract; however, dermal absorption is relatively low.
- 2,4-D distributes widely in tissues following oral exposure, but does not accumulate in tissues.
- 2,4-D is subject to limited metabolism.
- 2,4-D is relatively rapidly eliminated in the urine.
- The toxicokinetics of 2,4-D is species- and sex-dependent largely due to differences in renal clearance of 2,4-D. The differential capacity for excreting 2,4-D plays an important role in the susceptibility to 2,4-D-induced effects between species.

3.1.1 Absorption

No studies were located regarding absorption of 2,4-D following inhalation exposure.

Evidence of gastrointestinal absorption of 2,4-D in humans comes from analysis of 2,4-D in tissues and fluids from cases of intentional or accidental ingestion of commercial products containing 2,4-D that resulted in death and from studies with volunteers. Quantitative data are available from the latter studies.

Results from studies in volunteers have shown that oral absorption of 2,4-D in humans is rapid and virtually complete. For example, oral administration of a single dose of 5 mg/kg 2,4-D in a gelatin capsule to six male volunteers resulted in a significant amount of the compound in plasma 1 hour after dosing and in a maximum of approximately 30 µg/mL 7–24 hours after dosing (Kohli et al. 1974). Assuming first rates of absorption and clearance, the investigators estimated a plasma half-life of 33 hours. A similar study in which five male volunteers were administered 5 mg/kg analytical-grade 2,4-D reported that plasma levels achieved a maximum of 10–30 µg/g approximately 6 hours after dosing (Sauerhoff et al. 1977). Elimination from plasma appeared to follow a one-compartment model for two subjects and a one- or two-compartment model for the third subject. Two subjects were not modeled. The volumes of distribution for the former were 238 and 294 mL/kg, and 83 and 218 mL/kg for the third subject if a two-compartment model was assumed; these data suggested relatively limited distribution to

tissues. The pooled half-life value for clearance of 2,4-D from plasma was 11.6 hours. Based on recovery data, it was estimated that absorption ranged from 87.6 to 106.3% of the administered dose.

Oral absorption in animals is fast and complete, particularly at relatively low doses (≤50 mg/kg), as assessed by early detection of 2,4-D in tissues and almost complete recovery of the dose in urine (i.e., Khanna and Fang 1966).

Dermal absorption of 2,4-D in humans is low compared to oral absorption. Male volunteers who received a topical application of 4 μg/cm^2 of 2,4-D in acetone on the ventral forearm excreted only 5.8% of the applied dose in the urine over a 5-day monitoring period (Feldmann and Maibach 1974). The application site was not protected and the subjects were asked not to wash the site for 24 hours. These results are consistent with those from a similar study in male volunteers who reported that 4.5% of an applied dose of 10 mg 2,4-D in acetone/water over a 9 cm^2 area on the dorsum of the hand was absorbed over a 144-hour period (Harris and Solomon 1992). Using data from Feldmann and Maibach (1974) in an exponential saturation model with lag time, Thongsinthusak et al. (1999) estimated dermal absorption of 2,4-D in humans to be 21.2–21.7% of the applied dose. In a review of the literature, however, it was noted that because the results of Harris and Solomon (1992) indicated that excretion of 2,4-D was essentially complete by 144 hours, using models much beyond 120 hours will overpredict absorption (Ross et al. 2005), so the results of Thongsinthusak et al. (1999) are not reliable.

Based on recovery of 2,4-D in the urine, a comparative study showed that rabbits absorbed a higher percentage (36% of the dose) of the applied dose than monkeys and that absorption rate can vary with the application site (Moody et al. 1990). Monkeys absorbed almost twice the amount of 2,4-D when the compound was applied on the forehead (29% of the dose) than when applied on the forearm (15% of the dose). Another study in monkeys reported an absorption rate of 8.6% of the dose when 2,4-D in acetone was applied on the abdomen of the animals (Wester et al. 1996). Application of 2,4-D in soil onto a 12-cm^2 area of abdominal skin lightly clipped resulted in absorption of 9.8% of the dose when the soil load was 1 mg/cm^2 and 15.9% when the soil load was 40 mg/cm^2. Because the dose of 2,4-D applied was the same with both soil loads, the results showed that, under the conditions of the study, dermal absorption from soil was not significantly affected by soil load (Wester et al. 1996). However, a study with human skin *in vitro* in which the concentration of 2,4-D in soil was 5 ppm (5 mg 2,4-D/kg soil) reported that dermal absorption of 2,4-D was dependent on both soil load and the type of soil (Duff and Kissel 1996).

In a comparative study in which rats and guinea pigs were applied ^{14}C-2,4-D onto the skin, rats and guinea pigs absorbed a total of 49 and 40%, respectively, of the applied dose over a 14-day monitoring period (Moody et al. 1994). The value estimated for the rat in this *in vivo* study was consistent with a 40% absorption estimated in a dermatomed skin preparation *in vitro*, but not so for the guinea pig in which only 14% of a 2,4-D dose was absorbed through a skin preparation *in vitro*. For comparison purposes, 19% of a dose of 2,4-D in acetone was absorbed through human skin *in vitro* and 14% through pig skin *in vitro* (Moody et al. 1994). Approximately 2% of 2,4-D in soil was absorbed through human skin *in vitro* (Wester et al. 1996). However, when using acetone as a vehicle, 19% of an applied dose of 2,4-D was absorbed (Moody et al. 1994).

In mice, approximately 7% of a dose of 1 mg/kg of ^{14}C-2,4-D in acetone penetrated the body (disappeared from the covered site of application) in 1 hour and about 21% in 24 hours (Grissom et al. 1985).

A series of studies by Brand and coworkers (Brand et al. 2002, 2003, 2004, 2007a, 2007b) examined factors that can influence the dermal absorption of 2,4-D in animal models. Using hairless mice skin *in vitro*, the investigators reported that six out of nine commercially available sunscreens significantly increased the total penetration of 2,4-D through the skin over a 24-hour period (Brand et al. 2002). Total penetration of 2,4-D ranged from 39.1% for no sunscreen used to 81.0% for the sunscreen that facilitated penetration the most. Subsequent studies showed that ultraviolet (UV) absorbers in sunscreens significantly enhanced the transdermal absorption of 2,4-D (Brand et al. 2003; Pont et al. 2004). The investigators also showed that dietary exposure of rats to ethanol for 6–8 weeks resulted in increased penetration of 2,4-D through the rat skin in an *in vitro* diffusion system, most likely due to altering the properties of the dermal barrier, possibly by inducing changes in lipid peroxidation and increasing transepidermal water loss (Brand et al. 2004, 2007a). Results from an additional study showed that the combination of sunscreen use and ethanol ingestion enhanced penetration of 2,4-D in rats' skin in an additive manner (Brand et al. 2007b).

Analysis of plasma from rats following an intravenous injection of 5 mg/kg 2,4-D showed a significantly smaller volume of distribution in females (50.2 mL) than in males (80.6 mL), consistent with significantly higher plasma concentration of 2,4-D (Griffin et al. 1997a). In addition, clearance (mL/minute) was about 10-fold lower in females than in males, whereas elimination half-lives from plasma were significantly higher in females.

3.1.2 Distribution

No information was located regarding distribution of 2,4-D following inhalation exposure of humans or animals.

Distribution data for 2,4-D following oral ingestion by humans are available in case reports that resulted in death; the results showed wide distribution in tissues. For example, reports by Dudley and Thapar (1972), Nielsen et al. (1965), Osterloh et al. (1983), and Keller et al. (1994) showed measurable amounts of 2,4-D in all organs that were examined, including the brain, liver, kidney, spleen, muscle, body fat, pancreas, heart, and lungs.

Studies in animals have shown that 2,4-D is widely distributed in tissues after oral dosing. In a study in rats, some 2,4-D-derived radioactivity was detected in all 12 tissues examined as early as 1 hour after gavage dosing (Khanna and Fang 1966). Rats were given approximately 3 or 240 mg/kg 2,4-D. With the low dose, peak concentration in tissues was achieved 6–8 hours after dosing. Elimination was fast (half-life 0.58 hours), with no detectable radioactivity in tissues 24 hours after dosing. Aside from the stomach, the kidneys had the highest amount of radioactivity and the brain had the least; no radioactivity could be detected in the brain within the first 4 hours after dosing. In high-dose rats, peak concentrations in tissues occurred 8 hours after dosing and could still be detected in tissues 41 hours after dosing. Elimination half-lives ranged from 3 to 3.5 hours; the brain had the lowest amount of label at all times and the kidneys had the highest. Examination of the intracellular distribution of 2,4-D in the nuclear, mitochondrial, microsomal, and soluble fractions of the kidneys, liver, spleen, brain, heart, and lungs showed that all fractions contained significant radioactivity. Regardless of the dose, most radiolabel was found in the soluble and nuclear fractions, while the microsomal and mitochondrial fractions only contained 1.4–6.7% of the total radioactivity. Because the radioactivity in the soluble fraction from all tissues could easily be extracted with ether, Khanna and Fang (1966) suggested that the 2,4-D molecule in the soluble fraction was not protein- or peptide-bound.

A comparative study in rats, mice, and hamsters of both sexes showed that ^{14}C-2,4-D-derived radioactivity was widely distributed in tissues following a single oral dose (5 or 200 mg/kg) of 2,4-D, but differences between sexes were apparent in rats and hamsters (Griffin et al. 1997a). In general, over a 72-hour monitoring period, liver and kidneys appeared to have the most radioactivity at early time points (2–8 hours); skin and fat showed relatively high amounts of radioactivity throughout the monitoring period in animals given the high dose of 2,4-D. Tissues levels of radioactivity were consistently higher in

female rats than in male rats, although the differences were not always statistically different. In hamsters, tissue levels of radioactivity were more often than not higher in males than in females. No clear differences in disposition of radioactivity were established between male and female mice.

A study in rats showed that postnatal dietary maternal exposure to 2,4-D can result in transfer of 2,4-D to the offspring via the milk (Stürtz et al. 2006). Over a dose range of 15–70 mg/kg, the concentrations of 2,4-D in dams' serum, milk, and 16-day-old pups' serum were dose-dependent, but were significantly lower in pups' serum than in maternal media. The study also showed 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. More recently, Saghir et al. (2013a) also demonstrated excretion of 2,4-D in rat's milk following perinatal exposure to 2,4-D via the diet. On lactation day 4, the concentration of 2,4-D in milk was 1.7–6.3 times lower than the concentration in the dams' plasma. The ratio was reduced to 1.5–2.5 times lower on lactation day 14 due to an approximate doubling of the dams' intake of 2,4-D in the 10-day interval. The concentration of 2,4-D in pups' plasma also increased from PND 4 to 10. Over the range of dietary concentrations tested (10–1,600 ppm 2,4-D), the ratios of pups' plasma 2,4-D/maternal plasma 2,4-D increased greatly on PND 14 relative to PND 4.

No information was located regarding distribution of 2,4-D following dermal exposure of humans or animals. However, since dermal absorption occurs, it is reasonable to assume that 2,4-D will distribute in a manner similar to that reported in oral animal studies.

In adult male rats, subcutaneous administration of a dose of 250 mg/kg 2,4-D followed by intravenous dosing of radiolabeled 2,4-D resulted in most of the radiolabel in the plasma, kidneys, and liver about 2 hours after dosing (Elo and Ylitalo 1979). Somewhat lower amounts were reported in the lungs and heart, and significantly lower amounts were found in the brain, muscle, testes, and cerebral spinal fluid. In a study that only evaluated brain distribution, subcutaneous administration of 300 mg/kg 2,4-D (half the LD_{50}) followed by intravenous radioactive 2,4-D resulted in radioactivity widely distributed in various brain areas (cerebral cortex, striatum, medulla oblongata, cerebellum, and midbrain brain, including hippocampus, hypothalamus, and thalamus) without any one area showing preferential accumulation of radioactivity (Tyynelä et al. 1990). In adult rabbits, administration of a single intraperitoneal low dose of ^{14}C-2,4-D resulted in wide distribution of radioactivity throughout the brain 2 hours after dosing, and ranged from 2.8% of plasma in the hypothalamus to 4.58% in the brainstem (Kim et al. 1988).

Intravenously injected 2,4-D to pregnant mice tended to accumulate in the visceral yolk sac and after passing to the fetus, was eliminated from all tissues within 24 hours (Lindquist and Ullberg 1971). Another study in pregnant mice given an intraperitoneal injection of ^{14}C-2,4-D on GD 17 showed that 3 hours after dosing, radioactivity was distributed in various brain regions and ranged from a low of 2.8% of that of plasma in the caudate nucleus to 4.6% in the brainstem (Kim et al. 1988). Fetal brain as a whole contained 5.8% of the amount in plasma, suggesting that the brain barrier forms early in fetal life. Intravenous injection of ^{14}C-2,4-D to pregnant rabbits on GDs 28–30 resulted in rapid transfer of radioactivity to fetal plasma and brain (Sandberg et al. 1996). Peak concentrations of radiolabel were achieved in fetal plasma approximately 30 minutes after injection and remained relatively constant for the remainder of the 2-hour sampling period. Except for radiolabel in plasma, maternal kidneys and uterus showed the highest tissue AUCs. In maternal brain, lateral and ventricular choroid plexus had the highest concentration of radioactivity (about 10 times higher than any other brain region). Fetal brain had the lowest concentration of label of any maternal or fetal organ sampled. However, the concentration in fetal brain tissue was 7% of that in fetal plasma compared to 2% of that in maternal plasma, suggesting possible increased vulnerability of the fetus. In general, maternal and fetal tissue AUCs increased proportionally as the dose of 2,4-D increased from 1 to 10 mg/kg; however, in fetal tissues, it also increased 10-fold when the maternal dose increased from 10 to 40 mg/kg. The investigators suggested that because only unbound compound was available for placental transfer, the greater increase in fetal AUCs suggested saturation of maternal 2,4-D plasma protein binding (Sandberg et al. 1996).

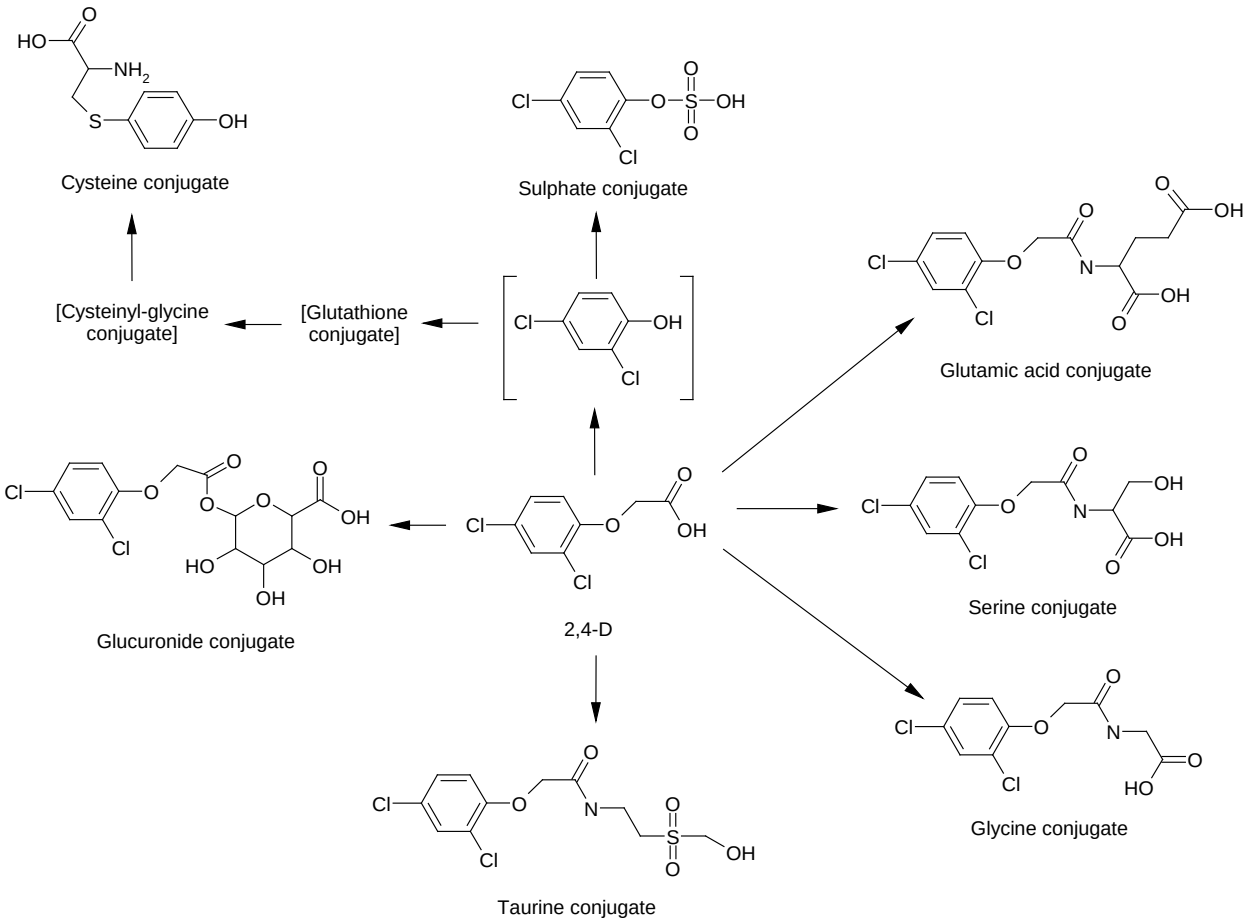
Transfer of 2,4-D to the offspring was also observed in rats following intraperitoneal injections to nursing dams every other day up to PND 16 (Stürtz et al. 2000). Transfer to 2,4-D was evident already in 4-day-old pups. In general, 2,4-D residues in pups' stomach contents, blood, kidney, and brain were dose- and exposure-time-dependent. The stomach content (milk) and the kidneys always contained the highest concentrations of 2,4-D. Levels of 2,4-D in kidneys in 8-day-old offspring from high-dose dams (100 mg/kg) increased 6-fold compared to 4-day-old pups. Pups' brain always had the lowest concentration of 2,4-D, which varied 10-fold between low-dose (50 mg/kg) 4-day-old pups and high-dose 16-day-old pups. The latter gained significantly less weight than controls, which the investigators attributed to diminished milk intake and/or a direct toxic effect of 2,4-D. Unlike Stürtz et al. (2006), these investigators discounted the quality of milk as a reason for less weight gain.

3.1.3 Metabolism

Studies in humans and animals have shown that 2,4-D undergoes limited metabolism in the body based on identification and quantification of products in the urine. For example, in a group of six male volunteers, only unchanged 2,4-D was detected in urine samples over a 1-week period after receiving a single oral dose of 5 mg/kg 2,4-D in a gelatin capsule (Kohli et al. 1974). In a similar study, analysis of urine samples from five volunteers following ingestion of 5 mg/kg 2,4-D showed mostly unchanged parent compound (mean 82.3% of the administered dose) with smaller amounts (mean 12% of the dose) excreted as a 2,4-D conjugate over a 6-day period (Sauerhoff et al. 1977).

Studies in animals have shown that, depending on the species, 2,4-D does not undergo metabolism, or if it does, as in dogs, it undergoes phase II metabolism to form conjugates that are excreted mainly in the urine; the biliary system plays only a minor role (Griffin et al. 1997b). Griffin et al. (1997a) studied the metabolism of 2,4-D in rats, mice, and hamsters and reported qualitative and quantitative differences in metabolite profiles between species, but not between sexes. Following administration of an oral dose of 5 or 200 mg/kg ^{14}C-2,4-D, the parent compound was the major urinary metabolite in the three species. A glycine conjugate was identified in the urine from mice and hamsters, a taurine conjugate was present in the urine from mice and male hamsters, and a glucuronide was detected only in urine from hamsters. Male mice metabolized 2,4-D to the glycine conjugate to a greater extent than female mice. A more recent comparative study in rats and dogs administered a single oral dose of 5 or 50 mg/kg ^{14}C-2,4-D reported that 2,4-D was excreted unmetabolized in the urine as parent compound (van Ravenzwaay et al. 2003). In dogs, however, 2,4-D formed taurine, serine, glycine, glutamic acid, cysteine, sulfate, and glucuronide conjugates, which were excreted in the urine; dog plasma only contained unchanged 2,4-D. In general, although conjugation is minimal, it favors elimination in the urine. Figure 3-1 shows a proposed metabolic pathway for 2,4-D in dogs.

Figure 3-1. Proposed Metabolic Pathway of 2,4-D in Dogs



2,4-D = 2,4-dichlorophenoxyacetic acid

Source: Van Ravenzwaay et al. 2003

3.1.4 Excretion

No data were located regarding elimination of 2,4-D in humans or in animals following inhalation exposure. However, 2,4-D has been measured in the urine of workers who experienced multi-route exposure, including inhalation (see Section 3.3.1 Biomarkers of Exposure).

In six healthy male volunteers administered a gelatin capsule with 5 mg/kg 2,4-D, unchanged compound was detected in the urine as early as 2 hours after ingestion; >75% of the parent compound was excreted in the urine in 96 hours (Kohli et al. 1974). A similar study with volunteers reported that most of a single

oral dose of 5 mg/kg 2,4-D was excreted unchanged in the urine within 3 days of dosing (Sauerhoff et al. 1977). Over a 6-day period after dosing, recovery of the administered dose was almost complete. The half-life elimination from urine ranged from 10.2 to 28.5 hours. The estimated fraction of the dose eliminated in the urine as free 2,4-D over the 6-day period ranged from 47.8 to 96.5%.

Studies in animals show that 2,4-D is eliminated mainly in the urine as unchanged compound or as conjugate, as it occurs in dogs.

In urine from rats collected every 10 hours after gavage administration of a single dose of 2.6 mg/kg 2,4-D as the sodium salt by gavage in water, peak concentration of 2,4-D occurred in the 20-hour spot sample (Knopp and Schiller 1992). Gradual decline occurred over the next 10 hours, and by 40 hours after dosing, approximately 90% of the administered dose had been accounted for in the urine. In an earlier study in rats administered doses of approximately 3–30 mg/kg ^{14}C-2,4-D by gavage, excretion of 2,4-D was virtually complete within 48 hours of dosing and 93–96% of the dose was excreted in the first 24 hours (Khanna and Fang 1966). Almost all of the radioactivity corresponded to parent compound and was excreted in the urine; no radioactivity could be detected in expired air. Administration of higher doses (~60–300 mg/kg) resulted in a linear decrease in recovery of radiolabel in urine and feces, and increased amounts were recovered in the second 24 hours after dosing. Excretion of the higher dose was still incomplete 144 hours after dosing.

In a comparative study in rats, mice, and hamsters administered a single dose of 5 or 200 mg/kg ^{14}C-2,4-D, urine was the main route of elimination of radiolabel in the three species (Griffin et al. 1997a). In rats, <4% of the administered radioactivity appeared in the feces during the 72-hour monitoring period. No 2,4-D metabolites were detected in the urine or feces from rats. Mice excreted 10–24% of administered radioactivity in the feces, and of this, 13.3% was the taurine conjugate. Hamsters excreted 6–16% of the administered radioactivity in the feces and all of it was unchanged 2,4-D. In the three species, expired air contained <1% of the administered radioactivity. In a similar study in rats and dogs administered 5 or 50 mg/kg ^{14}C-2,4-D, irrespective of the dose, rats excreted almost all of the administered radioactivity in the urine, and excretion was virtually complete 24 hours after dosing (van Ravenzwaay et al. 2003). Dogs metabolized 2,4-D (Figure 3-1). Low-dose dogs excreted approximately 38% of the dose in the urine and 10–13% in the feces over the 120-hour monitoring period. High-dose dogs excreted about equal amounts of the dose (20–25%) in the urine and feces. Excretion was not complete in dogs after the 120-hour sampling time. No significant differences regarding rates or routes of excretion between male and female animals were observed.

An oral exposure study provides suggestive evidence of sex-related differences in the elimination of 2,4-D from plasma (van Ravenzwaay et al. 2003). In rats administered 5 mg/kg, no differences in plasma elimination half-lives were observed between males and females. However, at 50 mg/kg, the elimination half-life was almost twice as long in the female rats (3.35 hours) than in the males (1.50 hours). In contrast, the plasma elimination half-times were similar between male and female dogs administered 5 or 50 mg/kg (van Ravenzwaay et al. 2003).

In volunteers applied a dermal dose of 4 μg/cm^2 2,4-D in acetone, most of the absorbed dose was eliminated in the urine within 72 hours of dosing (Feldmann and Maibach 1974). In a similar study, subjects applied a dose of 10 mg of 2,4-D in acetone over a 9 cm^2 area excreted most of the absorbed dose in 96 hours; an average of 84.8% of the applied dose was recovered in the urine in 96 hours. The approximate mean urinary excretion half-life was 39.5 hours (Harris and Solomon 1992).

Application of an aqueous solution of 2.6 mg/kg 2,4-D sodium salt to the shaved back of rats resulted in significantly lower urinary concentration of 2,4-D than when the dose was administered orally (Knopp and Schiller 1992). Peak urinary concentration of 2,4-D occurred at about 40 hours after dosing and declined gradually thereafter. As a percentage of the applied dose, 2,4-D in the urine increased steadily over a 116-hour period after dosing, reaching a cumulative maximum of about 10.5% of the applied dose. In rabbits, 36% of a dose of 4 μg/cm^2 of 2,4-D in acetone applied to the shaved back was recovered in the urine over a 14-day period (Moody et al. 1990). In the same study with monkeys and rabbits, 15 and 29% of the dose applied to the forearm and forehead, respectively, was recovered in the urine over the same time period. Urinary excretion half-lives ranged from 1.47 days for the monkeys' forehead application to 2.14 days for the rabbits' back application.

In rats, fecal excretion of ^{14}C-2,4-D represented only a minor elimination route following dermal application of the chemical, with only 2% of the applied dose accounted for in the feces over a 14-day sampling period (Moody et al. 1994). In the same time period, guinea pigs excreted 9% of a dermal dose of 2,4-D in the feces (Moody et al. 1994). Mice applied a dose of 1 mg/kg ^{14}C-2,4-D in acetone on the shaved back excreted small amounts of radiolabel in the feces and as CO_2, although the authors did not provide the specific amounts (Grissom et al. 1985). In 24 hours, 93% of 2,4-D that had penetrated the application site (almost 21% of the applied dose) was accounted for in the excreta.

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

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

PBPK models for 2,4-D in rabbits, rats, and humans have been reported (Durkin et al. 2004; Kim et al. 1994, 1995, 1996, 2001). The Kim et al. (1994, 1995, 1996, 2001) models were developed with the primary objective of simulating regional brain distribution of 2,4-D. These models included compartments for various brain regions, while all other tissues were aggregated into a single compartment. The rat and human models developed by Durkin et al. (2004) have compartments for liver and kidney, but no separate compartment for brain. The model has been applied to interspecies and route-to-route dosimetry calculations for deriving Hazard Quotients (ratio of a measure of exposure to a chemical to an established benchmark such as a reference dose [RfD] or reference concentration [RfC]) in forestry workers who spray 2,4-D based on dose equivalence for plasma peak and average 2,4-D concentrations. The Durkin et al. (2004) and Kim et al. (2001) models differ in several other important ways. In the Durkin et al. (2004) model, exchanges of 2,4-D between plasma and tissues are flow-limited with partitioning of the non-ionized species (e.g., protonated acid) between interstitial and intracellular fluid in tissues. Uptake of the anionic base is attributed to differences in extracellular and intracellular pH, which result in intracellular pH-trapping of the anionic base. In the Kim et al. (2001) models, exchanges between plasma and tissues are diffusion limited and no distinction is made between the protonated and anionic species. Another important difference concerns the simulation of urinary excretion of 2,4-D. In the Durkin et al. (2004) model, renal clearance of 2,4-D is dependent on plasma 2,4-D concentration, with renal clearance decreasing as plasma 2,4-D concentration increases. This approach accommodated results of studies in animals that found dose-dependent inhibition of urinary excretion of 2,4-D. In the Kim et al. (2001) model, urinary excretion is simulated as a capacity-limited transfer of 2,4-D to urine. Both models include a “deep” compartment, which exchanges 2,4-D with plasma very slowly. In the Durkin et al. (2004) model, the deep compartment is assigned to red blood cells; in the Kim et al. (2001) models, the deep compartment is assigned a subcompartment of the lumped

body compartment representing all tissues other than brain. The Durkin et al. (2004) model also includes parameters for simulating binding of 2,4-D to plasma protein. Although very different in structure, both models yielded similar predictions of plasma elimination kinetics when optimized to the same intravenous dosing studies in rats (Durkin et al. 2004).

Rabbit (Kim et al. 2001)

Kim et al. (1994, 1995, 1996, 2001) developed a PBPK model for predicting uptake and distribution of 2,4-D in rabbit and rat brain. The model includes compartments for plasma, brain, and a single lumped body compartment representing all tissues other than brain. The brain compartment includes subcompartments representing the hypothalamus, caudate nucleus, hippocampus, forebrain, brainstem, cerebellum, brain plasma, and cerebrospinal fluid (CSF). The six brain compartments have distinct mass transfer clearance coefficients for plasma-brain and brain-CSF. The body compartment includes a deep subcompartment and a compartment representing the rest of the body (excluding brain). Exchanges of 2,4-D between plasma and brain are simulated as four process: (1) flow-limited exchange between central plasma and brain plasma, governed by blood flow and the brain/plasma partition coefficient; (2) diffusion-limited exchange between plasma and brain tissue governed by a mass transfer clearance coefficient; (3) diffusion-limited exchange between brain tissue and CSF; and (4) capacity-limited transfer from CSF to plasma, representing transport through the choroid plexus, governed by a V_{max} and K_m. Exchange of 2,4-D between plasma and the rest of the body is flow-limited. Excretion of 2,4,-D is represented as capacity-limited transfer from the body compartment (V_{max}, K_m).

Partition coefficients for the rabbit model were estimated from tissue/plasma concentration ratios measured in rabbits following a single intraperitoneal dose of 40 or 100 mg/kg ^{14}C-2,4-D (Kim et al. 1995). These same values were used in the rat model. Transfer coefficients for the rabbit model were optimized with data from the same study (Kim et al. 1995). Transfer coefficients for the rat model were optimized with data on plasma and brain concentrations in rats following intravenous injection of 10, 50, or 150 mg/kg 2,4-D or subcutaneous implantation of osmotic mini-pumps that delivered 2,4-D doses of 1 or 10 mg/kg day (Patterson et al. 2000). The rabbit model was evaluated by comparing observed and predicted time courses for plasma, CSF, and brain region 2,4-D concentrations. Data for an individual rabbit is displayed in Kim et al. (1995), and these plots show time profiles that are similar to observations. The rat model predicted plasma and brain regions concentration of 2,4-D that were within ±2 standard deviations of the mean observations (Kim et al. 2001).

A maternal-fetal model was developed based on the rabbit model (Kim et al. 1996). The model includes placental and amniotic fluid compartments and fetal tissue compartments representing fetal CSF, fetal brain tissue, and fetal brain plasma. Exchanges between maternal plasma and placenta are flow-limited. Exchanges between fetal plasma and brain include the same four flow-limited, diffusion-limited, and capacity-limited processes as in the maternal model. 2,4-D in amniotic fluid undergoes diffusion-limited exchange with 2,4-D in the fetal body compartment and with the placenta. Transfer coefficients were optimized based on data from a study in which anesthetized pregnant rabbits received intravenous doses of 1, 10, or 40 mg/kg ^{14}C-2,4-D on GD 30. The study provided time-course data on 2,4-D in maternal and fetal plasma, amniotic fluid, and fetal brain. The optimized model predicted the dose-dependent time course for 2,4-D fetal and maternal plasma, amniotic fluid, fetal brain, and maternal brain regions.

Human and Rat (Durkin et al. 2004)

Durkin et al. (2004) developed a PBPK model of 2,4-D for predicting internal exposures resulting from ingestion exposures in rats and dermal exposures in humans. The model includes compartments for plasma, red blood cells, skin, kidney, liver, gastrointestinal tract, and a lumped compartment representing other tissues. The blood compartment includes a red cell compartment, which exchanges 2,4-D slowly with plasma (first order). The plasma compartment includes saturable binding to two classes of binding sites. The free unbound fraction exchanges with tissues. Exchanges of 2,4-D between plasma and tissues are flow-limited with partitioning of the non-ionized species (e.g., protonated acid) between interstitial and intracellular fluid in tissues. Dissociation of the acid into its anionic base is calculated based on the Henderson-Hasselbalch equation, pKa for 2,4-D (2.87), pH of interstitial fluid (7.0), and intracellular fluid (7.4). The lower intracellular pH favors intracellular trapping of the anion. The liver compartment includes a term for first-order transfer of 2,4-D into the gastrointestinal tract representing biliary secretion. Excretion of 2,4-D is simulated as four processes: (1) delivery of 2,4-D into tubule fluid from glomerular filtration; (2) saturable transport of the anionic base from plasma into kidney (V_{max}, K_m); (3) secretion of the anionic based from kidney into urine (first order); and (4) excretion of 2,4-D in tubule fluid into urine (first order). Studies conducted in animals have found that urinary excretion of 2,4-D is inhibited by increasing concentrations of plasma 2,4-D (Orberg 1980). Although the mechanism for this apparent self-inhibition in not understood, the inhibition affects both glomerular filtration and renal secretion of 2,4-D, suggesting that it may represent a vascular effect resulting in depression of glomerular filtration and/or renal blood flow (Durkin et al. 2004). The pharmacodynamics of inhibition of urinary excretion are represented in the model as an adjustment to parameters that govern glomerular filtration, transport from plasma into kidney, and secretion of 2,4-D into urine. The adjustment factor is a variable

that changes in value as a function of plasma 2,4-D concentration. Dependence of the adjustment factor on plasma 2,4-D concentration results in renal clearance of 2,4-D decreasing with increasing plasma 2,4-D concentration. The adjustment factor was empirically derived from animal studies (Orberg 1980). Absorption pathways in the model are from the gastrointestinal tract and skin surface. The gastrointestinal tract model includes compartments representing stomach lumen, gastrointestinal tract lumen (representing the tract distal to stomach), and gastrointestinal tract tissue. Absorption from the stomach and transfer to feces are first-order processes. 2,4-D deposited on skin is subject to first-order transfer to the environment (fugitive loss) or first-order absorption into skin tissue from where it can undergo flow-limited exchange with plasma.

The model was parameterized to simulate rats, and subsequently extrapolated to humans. The rat model was based primarily on intravenous and oral studies (Durkin et al. 2004). Rats were administered a single intravenous dose (5 or 90 mg/kg) or oral dose (10, 25, 50, or 150 mg/kg). A study conducted in goats was used to estimate the effects of 2,4-D dose on 2,4-D renal clearance and glomerular filtration (Orberg 1980). Protein binding parameters were based on data from studies conducted in rats (Ylitalo et al. 1990), goats (Orberg 1980), and bovine serum albumin (Kolberg et al. 1973). Partition coefficients were estimated from physical-chemical properties of 2,4-D and tissue composition (Poulin and Krishnan 1995) and adjusted based on measured values for brain/plasma (Kim et al. 1995). The rat model was initially optimized based on data from the rat intravenous study and then applied to the rat oral study to estimate values for gastrointestinal tract absorption parameters. By parameterizing the model to achieve decreasing renal clearance in association with increasing plasma 2,4-D concentrations, the model predicted the observed nonlinear dose-dependence of urinary excretion and plasma concentration as well as time-dependent changes in kinetics of 2,4-D removal from plasma and excretion in urine following dosing (Durkin et al. 2004).

The human model was optimized to data from studies conducted in humans (Feldmann and Maibach, 1974; Sauerhoff et al. 1977). In the Feldmann and Maibach (1974) study, urinary ^{14}C was measured following a single intravenous (tracer) dose of ^{14}C-2,4-D or dermal dose to the forearm (4 µg/cm^2). In the Sauerhoff et al. (1977) study, plasma levels and urinary excretion of 2,4-D were measured following a single oral dose of 2,4-D (5 mg/kg). Data from the human studies were used to optimize values for parameters controlling the absorption rate from the gastrointestinal tract, absorption rate from skin, V_{max} uptake to kidney, and k_e for urinary excretion. All other parameters were allometrically scaled from the rat model.

The human model was evaluated by comparing observed and predicted urinary excretion of 2,4-D in forestry workers who sprayed 2,4-D from backpack sprayers (Lavy et al. 1987). The study provided data on application rates and urinary excretion of 2,4-D over a 5-day period. Skin deposition rates were estimated from data contained in the Pesticide Handlers Exposure Database (Durkin et al. 2004). Predictions from the optimized model encompassed observed cumulative urinary excretion of 2,4-D.

The model was applied to an interspecies and route-to-route dosimetry extrapolation. The model was used to predict plasma 2,4-D concentrations corresponding to a rat NOAEL and LOAEL estimated from a 90-day feeding study (Durkin et al. 2004). Average and peak plasma concentrations in rats corresponding to the NOAEL were predicted to be 3.6 and 7.2 µM, respectively. Average (2-week) plasma 2,4-D concentrations in forestry workers were predicted to range from 1.4 to 7.3 µM and peak concentration were predicted to range from 2.5 to 13 µM

3.1.6 Animal-to-Human Extrapolations

As mentioned previously, it has been proposed that the dog might not be a relevant species for evaluation of human health risk because of the relatively low capacity to excrete 2,4-D (Timchalk 2004). The implication is that, at equivalent doses of 2,4-D, more 2,4-D will remain in plasma and potentially reach tissues in dogs than in other species, particularly at lower doses since clearance may become saturated in most species at higher doses. This was illustrated by van Ravenzwaay et al. (2003) who reported that equivalent doses of 5 and 50 mg 2,4-D/kg given to rats and dogs resulted in plasma 2,4-D AUCs 125- and 15-fold greater, respectively, in dogs than in rats.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic

makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to 2,4-D are discussed in Section 5.7, Populations with Potentially High Exposures.

Epidemiological studies of farming communities where 2,4-D has been used, which have included monitoring of children, have not provided convincing evidence of associations between 2,4-D and adverse health outcomes in children. For example, no significant association was found between 2,4-D and birth weight in the AHS (Sathyanarayana et al. 2010), birth defects in the Ontario Farm Family Health Study (Weselak et al. 2008), or birth defects and congenital anomalies in a study of pesticide applicators in the San Joaquin Valley of California (Yang et al. 2014). Studies of state-licensed, private pesticide applicators in Minnesota found a significant increase in birth defects among children conceived during the herbicide application season (Garry et al. 1996, 2002). However, chemical-specific analyses were not conducted.

Further evaluation of children born to participants in the Ontario Farm Family Health Study showed a significant increased risk of hay fever or allergies associated with maternal exposure to 2,4-D during pregnancy among male offspring, but not among female offspring (Weselak et al. 2007). No significant association was found between exposure to 2,4-D and asthma or persistent cough or bronchitis.

Studies of children from parent participants in the AHS did not find significant associations between 2,4-D exposure and NHL, Hodgkin's disease, or leukemia (Flower et al. 2004). In a study of exposure to 2,4-D in house dust in California, childhood leukemia was not associated with 2,4-D (Metayer et al. 2013).

Animal studies have shown that 2,4-D can be transferred to the offspring through the placenta and via the mother's milk and that it distributes widely in fetal or neonatal tissues (Lindquist and Ullberg 1971; Marty et al. 2013; Saghir et al. 2013a; Sandberg et al. 1996; Stürtz et al. 2000, 2006). Therefore, it seems reasonable to assume that the same could happen in humans.

As summarized in Section 2.17, Developmental Effects, studies in rodents have shown that, for the most part, adverse developmental effects (i.e., mainly reduced body weight in the offspring) occur at maternal dose levels that induced maternal toxicity, mainly reduced maternal weight during pregnancy. Reduced

offspring weight was reported in a study in rats administered a relatively low postpartum dose of 2.5 mg 2,4-D/kg/day (Stürtz et al. 2010). This was attributed to 2,4-D affecting the suckling-induced hormone release milk transfer to the litter. However, no such effect has been reported in other studies that exposed dams to considerably higher doses (approximately 29 mg 2,4-D/kg/day) for periods that included gestation and postpartum (Marty et al. 2013).

2,4-D has not been found to cause teratogenicity in animal studies (Charles et al. 2001; Marty et al. 2013; Schwetz et al. 1971).

Children may be exposed to 2,4-D during and after its use in residential and recreational areas, such as on lawns or park grasses. Children may also be exposed when swimming in bodies of water that have been treated with 2,4-D (EPA 2005a). Children who live with farmworkers may also be exposed to 2,4-D from the clothing, boots, or containers brought into the home by household residents after a workday and spray drift proximal to fields, forests, and orchards (Arcury et al. 2007).

In a biomonitoring study of exposure to 2,4-D in farm families with licensed applicators in Minnesota and South Carolina, 24-hour urine 2,4-D concentrations were collected 1 day before through 3 days after application (Alexander et al. 2007). For children 4–17 years old (n=53), the median urine 2,4-D concentrations pre-application and 1 day after application were 1.5 and 2.9 µg/L, respectively. At baseline, 2,4-D was detectable in the urine of 62% of the children. The mean urine 2,4-D concentration in children the day before application, the day of application, 1 day after application, 2 days after application, and 3 days after application were 1.4, 2.1, 3.6, 3.5, and 3.4 µg/L, respectively. Younger children, 4–11 years old, had higher median post-application urine 2,4-D concentrations than older children, 12–17 years old (6.5 compared to 1.9 µg/L). Exposure to children was determined to be primarily attributable to the level of contact with the application process, including their presence during mixing or application of 2,4-D. Another study was performed to measure the level of pesticide urinary metabolites in 60 farmworker children 1–6 years old in North Carolina from July through August 2004 (Arcury et al. 2007). 2,4-D was detected in 41.7% of the 60 urine samples collected, with a median concentration of 0.23 µg/g creatinine.

Nishioka et al. (2001) performed a study to determine exposure to 2,4-D to young children (ages 5–14 years) in air and on surfaces (floors, tabletops, and windowsills) inside single-story Midwestern residences both before and after lawn application. 2,4-D was detected in indoor air and on all surfaces after application. It was determined that the main transport routes of 2,4-D into the home were from the

homeowner applicator and by an active dog. No 2,4-D was detected in indoor air samples before application. The maximum indoor air concentrations during and after application were 17.7 and 10.8 ng/m^3, respectively. Post application, floor dust was concluded to be the major source of 2,4-D in the air, on tables, and on windowsills by resuspension. Postapplication floor dust concentrations ranged from approximately 1 to 200 µg/m2, compared to 0.2–1.0 µg/m2 for dust levels prior to application. The concentrations of 2,4-D measured in occupied homes postapplication on carpets, bare floors, tabletops, and windowsills were <0.1–228, <0.01–23, 0.3–27, and 0.5–22 µg/m^2, respectively. It was estimated that dietary ingestion was the main source of exposure for young children before lawn application of 2,4-D, but during postapplication periods, dietary ingestion (53%), nondietary ingestion (41%), and dermal penetration (4%) were the main pathways. Postapplication exposure levels from nondietary ingestion by contact with floors and contact with tabletops were estimated to be 1–10 and 0.2–30 µg/day, respectively, which are estimated to be about 10 times higher than levels before application. Dust samples collected from the homes of 513 subjects residing in Detroit, Michigan, the state of Iowa, Los Angeles, California, and Seattle, Washington had an arithmetic mean and geometric mean concentration of 2,422 and 419 ng/g, respectively (Colt et al. 2004). Seventy-eight percent of all of the samples tested were positive for 2,4-D. Samples collected in Iowa had the greatest geometric mean concentration of 2,4-D (1,512 ng/g), followed by Detroit (606 ng/g), Seattle (374 ng/g), and Los Angeles (87 ng/g).

NHANES uses biomonitoring to provide estimates of exposure to the civilian U.S. population. Chemicals and their metabolites are measured in subsets of participants aged 6–59 years old, meant to be a representative sample of the population. Urinary levels of 2,4-D in children 6–11 and 12–19 years old were measured in NHANES samples assessing exposure from years 1999–2010 (CDC 2015). For survey years 1999–2000 and 2001–2002, no geometric mean urinary concentration of the 2,4-D could be calculated because the proportion of results below the detection limit was too high to provide a valid result. These results are summarized in Tables 5-7 and 5-8 (CDC 2015). The results suggest that urinary levels of 2,4-D in children have remained relatively unchanged over the temporal period, but slightly higher levels have been observed in children as compared to adults.

In the Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study, the exposures of 135 preschool children and their adult caregivers to 2,4-D at their homes in North Carolina and Ohio were examined in 2000 and 2001 (Morgan et al. 2008). Monitoring was performed over a 48-hour period, and personal (hand wipes and food) and environmental (air, soil, and dust) samples were collected. 2,4-D was detected in all types of environmental samples, with the highest frequency in carpet dust samples at 83% (median concentration of 47.5 ng/g) and 98% (median

concentration of 156 ng/g) in North Carolina and Ohio, respectively. Detection frequencies in North Carolina and Ohio were 38 and 49% (maximum concentrations of 3.7 and 2.0 ng/m^3) for indoor air, 19 and 34% (maximum concentrations of 1.7 and 3.2 ng/m^3) for outdoor air, and 17 and 45% (maximum concentrations of 30.5 and 13.3 ng/g) for soil, respectively. Maximum concentrations of 2,4-D in personal exposure samples for children in North Carolina and Ohio were 0.04 and 0.1 ng/cm^2 for hand wipes and 4.4 and 20.2 ng/g for solid food, respectively. 2,4-D was detected in >85% of the total samples collected. The median 2,4-D urinary concentrations in children were 0.5 and 1.2 ng/mL in North Carolina and Ohio, respectively. Morgan et al. (2014) estimated the potential intakes of 2,4-D from different routes using data from 129 preschool children from North Carolina in the CTEPP study. The daily intake dose was calculated as 4.981 ng/kg/day, with the largest intake arising from dietary exposure (4.84 ng/kg/day).

In a study of urine collected from 197 children in Arkansas, 20% had detectable levels of 2,4-D, and the 95th percentile and maximum concentrations were reported as 3 and 9 µg/L, respectively (Hill et al. 1989).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 2,4-D are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for 2,4-D from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of

tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by 2,4-D are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

As mentioned in Section 3.1.3, Metabolism, 2,4-D undergoes limited metabolism in humans and can thus be measured as unchanged parent compound in body fluids and tissues from humans. Information regarding levels in human tissues is available from cases of acute intentional or accidental oral intoxication with commercial products that contained 2,4-D that resulted in deaths (i.e., Dudley and Thapar 1972; Keller et al. 1994; Nielsen et al. 1965; Osterloh et al. 1983). Tissue levels of 2,4-D determined in these and other case reports are typically not representative of occupational or environmental exposure to 2,4-D.

2,4-D can be readily measured in urine, and with the benefit of non-invasive collection procedure, urine is a widely used and accepted media to ascertain exposure to 2,4-D. Because 2,4-D is rapidly eliminated from the body (Kohli et al. 1974; Sauerhoff et al. 1977), urinary levels of 2,4-D reflect recent exposure, within days.

There are many reports that provide information regarding urinary levels of 2,4-D in workers, especially farmers and herbicide applicators, and in members of the general population. Providing detailed information from the extensive number of studies available is beyond the scope of this document, but pertinent data have been extracted from recent reviews (Burns and Swaen 2012; von Stackelberg 2013). Additional information on this topic is presented in Chapter 5.

Burns and Swaen (2012) noted that large studies designed to be representative of the United States (CDC 2009; population surveyed years 1999–2002) and Canadian (Health Canada 2010) populations (surveyed years 2007–2009) did not detect 2,4-D at the 50th percentile (<1 µg/L) (in 50% of the samples, the concentration of 2,4-D was below 1 µg/L of urine). In general, urinary levels of 2,4-D in groups of individuals considered bystanders varied from less than the limit of detection (0.2 µg/L) to 3 µg/L. Bystanders were individuals who did not mix, load, or apply 2,4-D, but who occasionally could have experienced greater exposure than the general population. These included spouses and children of applicators, and applicators of other herbicides. Levels of 2,4-D in the urine from individuals who experienced direct exposures, such as those who applied 2,4-D on crops, forests, and turf, as well as those involved in the manufacture of 2,4-D, varied greatly. Geometric means between 5 and 45 µg/L were reported for crop and forestry applicators; maximum levels varied from 410 to 2,500 µg/L 2,4-D among these groups. A highest maximum of 12,963 µg/L was reported in a study of German manufacturers in the 1980s (Knopp 1994). The wide ranges reported are not surprising considering the number of factors that can determine the extent of exposure, including type of application method, glove use, repairing equipment, size of the area treated, and personal hygiene practices. A study reported that these factors explained 16% of the between-worker variance and 23% of the within-worker variance of urinary 2,4-D concentrations (Bhatti et al. 2010), suggesting that other determinants remained unexplained. It is worth noting that urinary pH is an important determinant of 2,4-D urinary levels.

Knowing the urinary levels of 2,4-D is important to determine whether someone has been exposed to excessive amounts of 2,4-D. This information is particularly useful if it can be used to estimate an absorbed dose of 2,4-D that can be compared to exposure guidance values. For example, Mage et al. (2004) collected data on urinary creatinine concentration and excretion rate from 978 volunteer participants in NHANES, 1988–1994, computed for their age, gender, height, and weight and determined that none of the subjects were exposed to 2,4-D at a rate above the reference dose (RfD) of 5 µg/kg/day established by EPA (EPA 2005a). A number of assumptions were made in this exercise, including assuming that the subjects had a relatively constant intake of 2,4-D and a constant dietary intake of red meat, and that the tubular secretion transport mechanism was not saturated. Under these conditions, the body would excrete approximately constant amounts of 2,4-D and creatinine per day. A similar approach was used by Alexander et al. (2007) to estimate systemic doses in farm families using urine samples collected from the application day through the third day after application. Subjects were participants in the Farm Family Exposure Study, a study of licensed applicators in Minnesota and South Carolina. The geometric means systemic doses (µg/kg/day) were as follows: 2.46 for applicators, 0.8 for spouses, 0.22 for children (all ages included), 0.32 for children 4–11 years of age, and 0.12 for children ≥12 years

of age. Exposure to family members was determined primarily by the potential for direct contact with the application process or chemical, although for many spouses and most children, it is more likely to be due to indirect exposure (contamination of surfaces, drift from application areas, in household dust) than direct exposure. Some factors found to be predictive of exposure were use of gloves, size of application, and having to repair equipment. The estimated systemic dose for applicators is consistent with a value of 2.7 µg/kg/day estimated for applicators in a study of participant in the AHS (Thomas et al. 2010b). Scher et al. (2008) developed a simple pharmacokinetic model from 2,4-D urinary excretion data from the Farm Family Exposure Study to evaluate the feasibility of reconstructing absorbed dose of 2,4-D. The model was a one-compartment model with single first-order absorption and elimination rate constants that adequately described the pharmacokinetic disposition of 2,4-D in humans as reported in studies with volunteers (Feldmann and Maibach 1974; Harris and Solomon 1992; Kohli et al. 1974; Sauerhoff et al. 1977). The final analysis was conducted on data from 14 farmers, and the results showed that the model accurately simulated measured urinary output and adequately described the data at early and late time points.

More recent studies have examined the use of biomonitoring equivalents to assess whether exposure to 2,4-D exceeds levels of concern (Aylward and Hays 2015; Aylward et al. 2010, 2013; Hays et al. 2012). Studies included both general population adults and children as well as farmers and farm family members. Biomonitoring equivalents are defined as a concentration of a chemical or its metabolite in a human biological medium (usually blood or urine) that is consistent with existent exposure guidance values (i.e., RfDs). The results of these studies showed that current exposures to 2,4-D are well below exposure guidance values for 2,4-D.

3.3.2 Biomarkers of Effect

Adverse effects, including death, have been observed in humans who intentionally or accidentally ingested herbicide formulations containing 2,4-D. Adverse effects were also reported following cases of accidental dermal exposure to 2,4-D. Some reported effects included tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects characterized by sensory and motor abnormalities. None of these conditions is specific for 2,4-D; any of these effects or combination of them can be caused by exposure to other chemicals or can be due to conditions unrelated to chemical exposures.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Limited information was located regarding interactions of 2,4-D with other chemicals. 2,4-D was found to increase the expression of some CYP1 cytochromes in rat liver, kidney, and mammary gland (Badawi et al. 2000) and of some microsomal enzymes in the liver of mice (Chaturvedi et al. 1991) and rats (Hietanen et al. 1983), and decrease some phase II enzymes in rat liver (Hietanen et al. 1983). This suggests that, in general, the toxicity of chemicals that are metabolized by the affected enzymes will increase or decrease depending on whether metabolism produces a reactive intermediate or a detoxification product. In general, in mice, 2,4-D combined with toxaphene seemed to have additive effects regarding microsomal enzyme induction and liver toxicity; the same, but to a lesser extent, occurred with the combination 2,4-D and parathion (Chaturvedi et al. 1991; Kuntz et al. 1990). Given that exposure to 2,4-D could coexist with exposure to other pesticides, more information on potential interactions would be useful.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

2,4-D is a free acid, phenoxy herbicide belonging to the phenoxyacetic acid chemical family, which is widely used in the United States. While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D (EPA 2005a). Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester. Almost 90–95% of total 2,4-D global use is accounted for by the dimethyl amine salt and ethylhexyl ester (Charles et al. 2001).

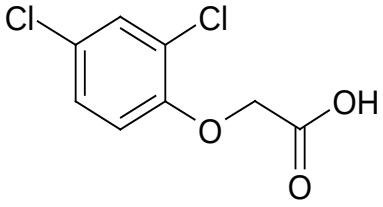
Formulations of 2,4-D and its derivatives vary in their chemical properties and behavior in the environment. However, most quantified analyses of 2,4-D and its derivatives are expressed in terms of the free acid (EPA 2005a).

Information regarding the chemical identity of 2,4-D and its derivatives are provided in Tables 4-1 and 4-2, respectively.

Table 4-1. Chemical Identity of 2,4-D[a]

Characteristic	Information
Chemical name	2,4-Dichlorophenoxyacetic acid
Synonym(s)	2,4-D; 2,4-D Acid; Acetic acid, (2,4-dichlorophenoxy)-
Registered trade name(s)[b]	Aqua-Kleen; Citrus Fix; Pyresta; Cimarron; Restore; Rush 24; 240; AMINO; Amoxone; Chloroxone; Crop Rider; Dinoxol; Dormone; Emulsamine; Fernimine; Fernoxone; Gesapax-H; Rilof-H; Target; Arena; Campeon; Fenix; Fenix Gold; Stockton; Talion; Turuna; Valsamba; Valsamin; Barrage; Brush-Rhap; Double Up; EndRun; HardBall; Opti-Amine; Trump-Card; Unison; Broadrange; Foundation; Weco Max; Brash; Phenoxy 088; Rugged; Strike; Charge; Dacomin; Chaser; Clean amine; Colt; Crossbow; Rifle; Saber; Salvo; Savage; Shotgun; Whiteout; Defy; Dical; Harvade; Willomine; Duplosan; Dyvel; Lotus; Topshot; U 46; Weedmaster; Speed-Mix; Gen-Amin; Gen-Ester; Grotex Complex; Grox; Trago

Table 4-1. Chemical Identity of 2,4-D[a]

Characteristic	Information
Chemical formula	$C_8H_6Cl_2O_3$
Chemical structure	
CAS Registry Number	94-75-7

[a]All information obtained from HSDB (2015), unless otherwise noted.
[b]Meister et al. 2014.

2,4-D = 2,4-dichlorophenoxyacetic acid; CAS = Chemical Abstracts Services; HSDB = Hazardous Substance Data Bank

Table 4-2. Chemical Identity of 2,4-D Derivatives

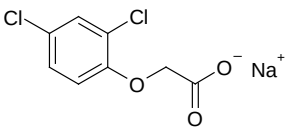
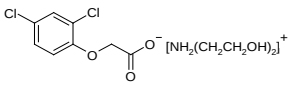
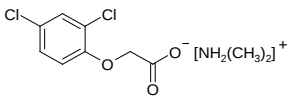
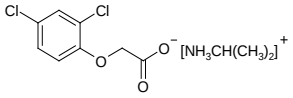
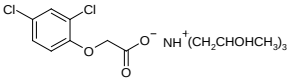
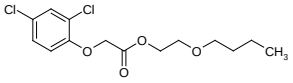
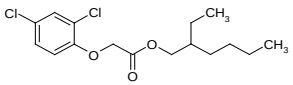
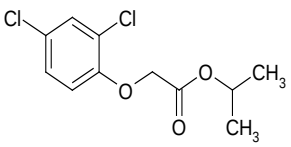
Characteristic	Information[a]		
Chemical name	2,4-D Sodium[b]	2,4-D Diethanolamine[c]	2,4-D Dimethylamine
Synonym(s)	Acetic acid, (2,4-dichlorophenoxy)-, sodium salt; Sodium 2,4-dichlorophenoxy-acetate; 2,4-Dichlorophenoxy-acetic acid, sodium salt; 2,4-D Na[b]	2,4-Diolamine; Acetic acid, (2,4-dichlorophenoxy)-, diethanolamine salt; 2,4-D Bis(2-hydroxyethyl) ammonium; 2,4-D DEA[b,c]	Acetic acid, (2,4-dichlorophenoxy)-, dimethylamine (1:1); (2,4-Dichlorophenoxy) acetic acid dimethylamine salt; Dimethylammonium (2,4-dichlorophenoxy) acetate; 2,4-D DMA
Registered trade name(s)	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1
Chemical formula	$C_8H_5Cl_2O_3.Na$[b]	$C_8H_6Cl_2O_3.C_4H_{11}NO_2$[b]	$C_8H_6Cl_2O_3.C_2H_7N$
Chemical structure[c]			
CAS Registry Number	2702-72-9	5742-19-8	2008-39-1

Table 4-2. Chemical Identity of 2,4-D Derivatives

Characteristic	Information[a]		
Chemical name	2,4-D Isopropylamine[c]	2,4-D Triisopropanolamine[c]	2,4-D Butoxyethyl ester
Synonym(s)	2,4-D-isopropylammonium; Acetic acid, (2,4-dichlorophenoxy)-, isopropylamine salt; 2-Propanamine, (2,4-dichlorophenoxy) acetate; 2,4-D IPA[b,c]	Acetic acid, (2,4-dichlorophenoxy)-, triisopropanolamine salt; 2,4-D-tris(2-hydroxypropyl) ammonium; 2-Propanol, 1,1',1''-nitrilotris-, (2,4-dichlorophenoxy) acetate; 2,4-D TIPA[b,c]	Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester; 2,4-Dichlorophenoxy-acetic acid, butoxyethyl ester; 2,4-D BEE
Registered trade name(s)	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1
Chemical formula	$C_8H_6Cl_2O_3.C_3H_9N$[b]	$C_8H_6Cl_2O_3.C_9H_{21}NO_3$[b]	$C_{14}H_{18}Cl_2O_4$
Chemical structure[c]			
CAS Registry Number	5742-17-6	32341-80-3	1929-73-3
Chemical name	2,4-D Ethylhexyl ester	2,4-D Isopropyl ester	
Synonym(s)	Isooctyl(2-ethylhexyl) 2,4-dichlorophenoxy-acetate; 2,4-D, 2-ethylhexyl; 2-Ethylhexyl (2,4-dichlorophenoxy) acetate; Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester; 2,4-D EHE	Acetic acid, (2,4-dichlorophenoxy)-, isopropyl ester; Acetic acid, (2,4-dichlorophenoxy)-, 1-methylethyl ester; 2,4-Dichlorophenoxyacetic acid isopropyl ester; Isopropyl (2,4-dichlorophenoxy)acetate; Isopropyl 2,4-D ester; 2,4-D IPE	
Registered trade name(s)	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1	
Chemical formula	$C_{16}H_{22}Cl_2O_3$	$C_{11}H_{12}Cl_2O_3$	
Chemical structure[c]			
CAS Registry Number	1928-43-4	94-11-1	

[a]All information obtained from HSDB (2015), unless otherwise noted.
[b]Meister et al. 2014.
[c]EPA 2005a.

2,4-D = 2,4-dichlorophenoxyacetic acid; CAS = Chemical Abstracts Services; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of 2,4-D and its derivatives are provided in Tables 4-3 and 4-4, respectively.

Table 4-3. Physical and Chemical Properties of 2,4-D[a]

Property	Information
Molecular weight	221.03
Color	White to yellow
Physical state	Crystalline powder
Melting point	138°C
Boiling point	160°C (at 4 mm Hg)
Density/specific gravity:	
at 25°C	1.42
Odor	Odorless; slightly phenolic
Odor threshold	3.13 mg/kg
Solubility:	
Water at 20°C	540 mg/L
Water at 25°C	677 mg/L
Organic solvents at 20°C:	
Ethanol	1,250 g/kg
Diethyl ether	243 g/kg
Heptane	1.1 g/kg
Toluene	6.7 g/kg
Xylene	5.8 g/kg
Octanol	120 g/L (25°C)
Partition coefficients:	
Log K_{ow}	2.81
Log K_{oc}	19.6–135.7
Vapor pressure at 20°C	1.40×10^{-7} mm Hg
Henry's law constant at 20°C	9.75×10^{-8} atm-m^3/mol
Autoignition temperature	No data
Flashpoint	Not combustible
Flammability limits	No data
Conversion factors	No data
Explosive limits	No data

[a]All information obtained from HSDB (2015).

2,4-D = 2,4-dichlorophenoxyacetic acid

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Sodium	2,4-D Diethanolamine
Molecular weight	243.03[b]	326.18[b]
Color	White[b]	Cream[b]
Physical state	Powder[b]	Powder[b]
Melting point	200°C[c]	83°C[c]
Boiling point	No data	No data
Density: at 25°C	42.2 pounds/feet^3 (0.676 g/cm^3) (bulk)[c]	0.762 g/cm^3 (bulk)[c]
Odor	No data	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	4.5x10^4 mg/L (unbuffered solution)[b]	8.06x10^5 mg/L (unbuffered solution)[b]
Organic solvents	No data	No data
Partition coefficients:		
Log K_{ow}	Not applicable[b,d]	0.0224–1.65[b]
Log K_{oc}	No data	No data
Vapor pressure at 25°C	Not applicable[b,d]	9.98x10^{-8} mm Hg[b]
Henry's law constant at 25°C	No data	No data
Autoignition temperature	No data	No data
Flashpoint	No data	No data
Flammability limits	No data	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Dimethylamine	2,4-D Isopropylamine
Molecular weight	266.1	280.04[b]
Color	White (pure); amber (technical)[b]	Amber[b]
Physical state	Crystals (pure); aqueous liquid (technical)[b]	Aqueous liquid[b]
Melting point	85–87°C	121°C[c]
Boiling point	Decomposition	No data
Density/specific gravity: at 20°C	1.23[c]	1.15[c]
Odor	Odorless	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	$3.0x10^6$ g/mL (20°C)	$1.74x10^5$ g/mL (pH 5) $4.36x10^5$ g/mL (pH 7) $3.31x10^5$ g/mL (pH 9) (unbuffered solutions)[b]
Organic solvents	Soluble in methyl, ethyl, and isopropyl alcohols, and acetone; insoluble in kerosene and diesel oil	No data
Organic solvents at 20°C		
Acetonitrile	1.06 g/100 mL	
Methanol	>50 g/100 mL	
Toluene	0.165 g/100 mL	
n-Hexane	0.00357 g/100 mL	
Octanol	5.41 g/100 mL	
Partition coefficients:		
Log K_{ow}	0.65	Not applicable[b,d]
Log K_{oc}	1.85–2.13	No data
Vapor pressure at 25°C	1 $x10^{-7}$ mm Hg[b]	Not applicable[b,d]
Henry's law constant at 25°C	$1.4×10^{-16}$ atm-m^3/mol[b]	No data
Autoignition temperature	No data	No data
Flashpoint	No data	No data
Flammability limits	Not flammable	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Triisopropanolamine	2,4-D Butoxyethyl ester
Molecular weight	412.31[b]	321.2
Color	Amber[b]	Amber; colorless
Physical state	Aqueous liquid[b]	Liquid
Melting point	87–110°C[c]	<25°C
Boiling point	No data	89°C[c]
Density/specific gravity: at 20°C	1.21	1.232 g/cm^3
Odor	No data	Odorless (pure); fuel oil-like (technical)
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	4.61x10^5 g/mL (pH 5) 4.61x10^5 g/mL (pH 7) 1.04x10^5 g/mL (pH 9) (unbuffered solutions)[b]	12 mg/L
Organic solvents	No data	Miscible in acetone, acetonitrile, n-hexane, and methanol; soluble in oils
Partition coefficients:		
Log K_{ow}	Not applicable[b,d]	4.1[b]
Log K_{oc}	No data	No data
Vapor pressure at 25°C	Not applicable[b,d]	4.5x10^{-6} mm Hg
Henry's law constant at 25°C	No data	No data
Autoignition temperature	No data	No data
Flashpoint	No data	>79°C (open cup)
Flammability limits	No data	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Ethylhexyl ester	2,4-D Isopropyl ester
Molecular weight	333.28	263.12
Color	Golden yellow	Colorless
Physical state	Liquid	Liquid
Melting point	<-37°C	5–25°C
Boiling point	>300°C (decomposition)	240°C[c]
Density: at 20°C at 25°C/25°C	 1.148 No data	 No data 1.255–1.270
Odor	Sweet, slightly pungent	Fuel oil-like (technical)
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	0.086 mg/L	37.3 mg/L
Organic solvents	No data	Soluble in alcohols and most oils
Partition coefficients:		
Log K_{ow}	5.78	253.8[c]
Log K_{oc}	No data	2.78[b]
Vapor pressure at 25°C	$3.6x10^{-6}$ mm Hg[b]	$2.32x10^{-4}$ mm Hg
Henry's law constant at 25°C	$1.8×10^{-5}$ atm-m^3/mol	$2.2×10^{-6}$ atm-m^3/mol[b]
Autoignition temperature	No data	No data
Flashpoint	171°C (open cup)	>79°C (open cup)
Flammability limits	No data	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

[a]All information obtained from HSDB (2015), unless otherwise noted.
[b]NPIC 2008.
[c]EPA 2005a.
[d]The salt dissociates to acid in water; therefore, this endpoint does not apply.

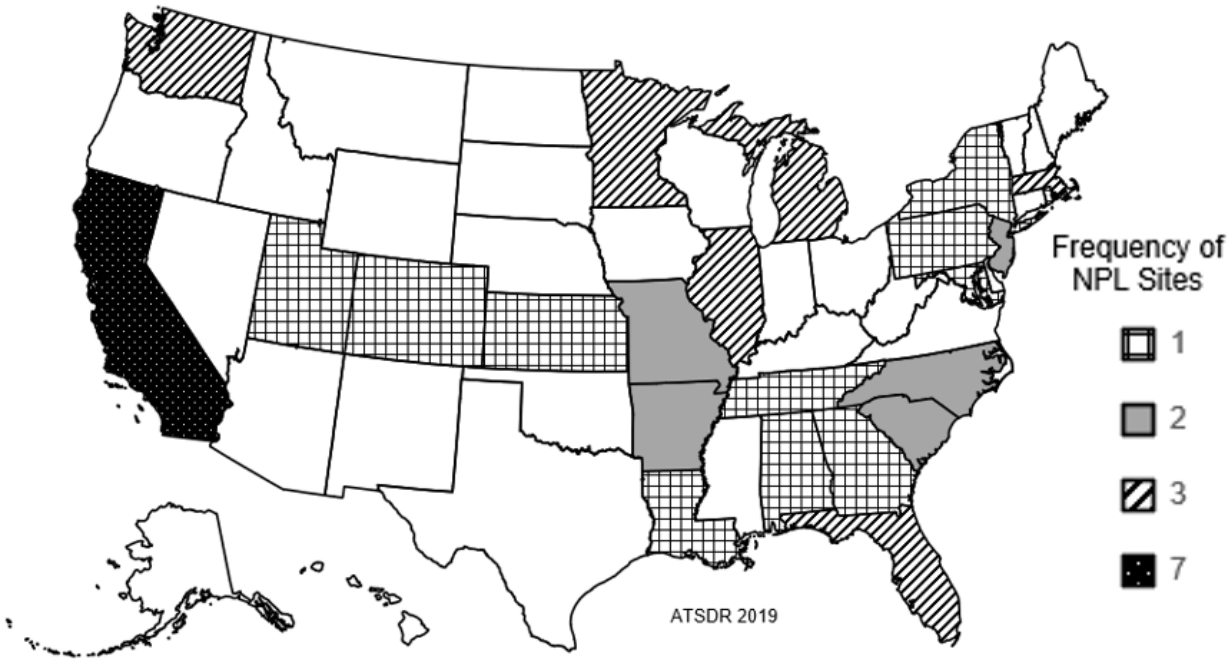
2,4-D = 2,4-dichlorophenoxyacetic acid

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

2,4-D has been identified in at least 46 of the 1,867 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2019). However, the number of sites in which 2,4-D has been evaluated is not known. The number of sites in each state is shown in Figure 5-1.

Figure 5-1. Number of NPL Sites with 2,4-D Contamination



- People can be exposed to 2,4-D through:
  - Inhalation of contaminated air
  - Consumption of food sources that contain 2,4-D residue
  - Dermal contact with 2,4-D during its application or by entering an area where it was very recently applied

- Environmental exposure of 2,4-D can occur:
  - During manufacturing processes 2,4-D
  - During or following application of 2,4-D to control weeds

- In the environment, 2,4-D is expected to:
  - Oxidize in the atmosphere or be removed by wet or dry deposition
  - Undergo photolysis in sunlit water and metabolism by aerobic bacteria
  - Readily undergo biodegradation and photodegradation from soil

2,4-D is one of the most widely used agricultural herbicides in the United States, with approximately 38 million pounds applied to crops in 2014, with pasture and hay fields, wheat, soybeans, and corn crops receiving the greatest applications (USGS 2016). It is also applied to residential or commercial turf for the elimination of a wide variety of broadleaf weeds without causing harm to the grass. Direct applications to rivers or lakes are occasionally made to control certain aquatic plants such as water chestnut or milfoil. Most forms of 2,4-D that are used today are supplied as the dimethyl amine salt (2,4-D DMA) or the ethylhexyl ester (2,4-D EHE).

In the atmosphere, 2,4-D is expected to exist in both the vapor and particulate phase. Vapor-phase 2,4-D is degraded by reaction with photochemically generated hydroxyl radicals with an estimated half-life of about 19 hours (Meylan and Howard 1993). Particulate-phase 2,4,-D is removed from the atmosphere by wet and dry deposition. Atmospheric levels of 2,4-D are generally very low, but detectable levels may be present in agricultural areas where 2,4-D has been applied as an herbicide (WHO 2003).

2,4-D may enter rivers, lakes, and ponds from spray drift following its aerial application or from runoff and erosion of soils treated with 2,4-D. It may also be directly applied to water surfaces in order to eradicate nuisance aquatic plants (Eyres 2009). The aerobic aquatic metabolism half-life of 2,4-D was reported to be about 15 days; however, it was more persistent in anaerobic aquatic metabolism studies, with a half-life ranging from about 41 to 333 days (EPA 2005a). Photolysis in sunlit surface waters may also be an important environmental fate process for 2,4-D, but hydrolysis under environmental conditions is expected to be negligible. Volatilization from water surfaces is not expected to be an important environmental fate process since 2,4-D salts do not volatilize. A bioconcentration factor (BCF) of 1, measured in carp, suggests that bioconcentration in aquatic species is expected to be low (NITE 2010a).

Field dissipation studies conducted in seven states over a 2-year period suggest that 2,4-D is not highly persistent in soils, with half-lives typically ranging from a few days to a few weeks depending upon the soil properties, water content of the soil, and whether 2,4-D was applied as a liquid or granular formulation (Wilson et al. 1997). The EPA reported that the biodegradation half-life of 2,4-D in an aerobic mineral soil was 6.2 days and the photodegradation half-life in soil was 68 days (EPA 2005a). Organic carbon normalized soil adsorption coefficients (K_{oc}) values of 70, 76, 59, and 117 using a sandy loam, sand, silty clay loam, and loam soil, respectively, suggest that adsorption to soil surfaces is low (EPA 2005a). Even though 2,4-D is expected to have high mobility in soils, its ability to leach into groundwater may be attenuated by its relatively short half-life in soils.

The general population is exposed to 2,4-D through both its agricultural and residential use. Ingestion of food and water contaminated with small residues of 2,4-D may occur for the general population. Persons residing within or very near areas of heavy 2,4-D use (e.g., farms) would have had an increased risk of exposure to greater amounts of 2,4-D through dermal contact with contaminated plants, soils, or surface waters or by inhalation from the applied herbicide. Those likely to receive the highest exposures are those who are involved in the production, formulation, handling, and application of 2,4-D. Dermal contact appears to be the major route of exposure for workers, although inhalation exposure and accidental ingestion via hand-to-mouth activity is possible. 2,4-D was detected in indoor air and on surfaces (floors, tabletops, and windowsills) inside single-story Midwestern residences following lawn applications (Nishioka et al. 2001). It was determined that the main transport routes of 2,4-D into the home were from the homeowner applicator and by pets.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

2,4-D is an herbicide belonging to the phenoxyacetic acid chemical family (NPIC 2008). It is produced by the reaction of 2,4-dichlorophenolate with monochloroacetic acid or by the reaction between 2,4-dichlorophenol and chloroacetic acid in aqueous sodium hydroxide (HSDB 2015). 2,4-D is sold commercially in the following formulations: emulsifiable concentrate, wettable granules, wettable powder, emulsion (esters), and aqueous solution (salts) (Meister et al. 2014).

Annual production of 2,4-D in the United States was estimated to be 52–67 and 47 million pounds in 1990 and 2001, respectively. Production in the United States was said to be between 50 and <100 million pounds in 2006 according to the EPA's Inventory Update Rule (IUR) (HSDB 2015). The EPA has replaced the IUR with the Chemical Data Reporting (CDR) Rule, which requires manufacturers (including importers) to give EPA nonconfidential information on the chemicals that they manufacture domestically or import into the United States. Data from the CDR lists only one producer of 2,4-D in the United States (the Dow Chemical Company), which declared their production volume as confidential business information for 2012 (EPA 2015a).

2,4-D is a chemical that manufacturing and processing facilities would be required to report under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986 [SARA]) (EPA 2005b). Table 5-1 lists the production

year, number of facilities, the state where each facility is located, and the range (in pounds) for each domestic manufacturer that reported production or formulation of 2,4-D in 2014 (TRI13 2015). Manufacturers are required to report Toxics Release Inventory (TRI) data to satisfy EPA requirements. The TRI data should be used with caution since only certain types of facilities are required to report (EPA 2005b). This is not an exhaustive list.

Table 5-1. Facilities that Produce, Process, or Use 2,4-D

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AR	1	100,000	999,999	9, 12
CA	1	10,000	99,999	7, 9
GA	1	1,000,000	9,999,999	7, 9
IA	2	100,000	9,999,999	7
IL	3	1,000	999,999	1, 2, 3, 4, 6, 7, 12
IN	1	Not available	Not available	Not available
KS	2	1,000,000	9,999,999	2, 3, 6, 7
MI	3	0	9,999,999	1, 3, 4, 6, 9, 12
MO	1	1,000,000	9,999,999	2, 3, 4, 6, 7
MS	1	100,000	999,999	7
MT	1	1,000,000	9,999,999	6, 7
NE	1	1,000	9,999	12
OH	4	100	999,999	7, 12
PA	1	Not available	Not available	Not available
TN	1	1,000,000	9,999,999	6, 7, 8, 9
TX	2	1,000	999,999	12
UT	1	100,000	999,999	9, 12
WI	1	Not available	Not available	Not available

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI16 2018 (Data are from 2016)

5.2.2 Import/Export

No current information regarding the import or export of 2,4-D could be located.

5.2.3 Use

While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D (EPA 2005a). Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester. Almost 90–95% of total 2,4-D global use is accounted for by the dimethyl amine salt and ethylhexyl ester (NPIC 2008). 2,4-D and its different chemical forms are listed as an ingredient, either as the singular active ingredient or in conjunction with other ingredients, in about 600 agricultural and residential products (EPA 2005a). The use of 2,4-D ranks first among herbicides in frequency of home and garden applications and third in national herbicide use for agriculture (Gilliom et al. 1999).

2,4-D is sometimes confused with the similarly named chemical, 2,4,5-T, which at one point in time was contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin, or TCDD, a confirmed toxin (CDC 2013). However, TCDD has never been a known contaminant of 2,4-D.

2,4-D is used on a wide range of broadleaf and aquatic weeds (EPA 2005a). Registered uses for 2,4-D can be seen in Table 5-2. These uses include application on field, fruit, and vegetable crops, as well as eliminating broadleaf weeds in turf, commercial and residential lawns while not harming the grass, and aquatic and forestry applications. The Midwest, Great Plains, and Northwestern United States have the most 2,4-D usage (EPA 2005a).

Table 5-2. Registered Uses for 2,4-D

Crop grouping	Representative crops
Terrestrial food crop	Pear, pistachio, stone fruits
Terrestrial food and feed crop	Agricultural fallow/idleland; agricultural rights-of-way/fencerows/ hedgerows; agricultural uncultivated areas; apple; barley; citrus fruits; corn (unspecified); corn, field; corn, pop; corn, sweet; fruits (unspecified); grapefruit; lemon; oats; orange; pome fruits; rice; rye; small fruits; soil, preplant/outdoor; sorghum (unspecified); soybeans (unspecified); sugarcane; tangelo; tree nuts; wheat
Terrestrial feed crop	Grass forage/fodder/hay; pastures; rangeland; rye; sorghum

Table 5-2. Registered Uses for 2,4-D

Crop grouping	Representative crops
Terrestrial nonfood crop	Agricultural fallow/idleland; agricultural rights-of-way/fencerows/ hedgerows; agricultural uncultivated areas; airports/landing fields; Christmas tree plantations; commercial/industrial lawns; commercial/institutional/industrial, premises/equipment (outdoor); forest nursery plantings (for transplant purposes); golf course turf; grasses grown for seed; industrial areas (outdoor); nonagricultural outdoor buildings/structures; nonagricultural rights-of-way/fencerows/ hedgerows; nonagricultural uncultivated areas/soils; ornamental and/or shade trees; ornamental lawns and turf; ornamental sod farm (turf); ornamental woody shrubs and vines; paved areas (private roads/sidewalks); potting soil/topsoil; recreation area lawns; recreational area; soil, preplant/outdoor; urban areas
Terrestrial nonfood and outdoor residential	Fencerows/hedgerows; nonagricultural rights-of-way/fencerows/ hedgerows; ornamental and/or shade trees; ornamental lawns and turf; ornamental woody shrubs and vines; paths/patios; paved areas (private roads/sidewalks); urban areas
Aquatic food crop	Agricultural drainage systems; aquatic areas/water; commercial fishery water systems; irrigation systems; lakes/ponds/reservoirs (with human or wildlife use); rice; streams/rivers/channeled water; swamps/ marshes/wetlands/stagnant water
Aquatic nonfood outdoor	Aquatic areas/water; streams/rivers/channeled water; swamps/ marshes/wetlands/stagnant water
Aquatic nonfood industrial	Drainage systems; industrial waste disposal systems; lakes/ponds/ reservoirs (without human or wildlife use)
Forestry	Conifer release; forest plantings (reforestation programs) (tree farms, tree plantations, etc.); forest tree management/forest pest management; forest trees (all or unspecified); forest trees (hardwoods, broadleaf trees); pine (forest/shelterbelt)
Outdoor residential	Residential lawns
Indoor nonfood	Commercial transportation facilities-nonfeed/nonfood

Source: EPA 2005a

2,4-D has been used in the United States since the 1940s (EPA 2005a). Due to some human health concerns, 2,4-D was placed in pre-Special Review by the EPA in 1986. In 1988, it was proposed that Special Review not be initiated due to the lack of epidemiological data linking 2,4-D and carcinogenicity and the final decision was deferred until reregistration. Between 1988 and evaluation for reregistration in 2005, the EPA performed several reviews of epidemiological and other data and still found that none of the new data definitively linked 2,4-D to human cancer cases. In order to address future concerns about its safety, the 2,4-D Industry Task Force agreed to certain changes to labeled uses to reduce exposure. In 2005, the EPA drafted its Reregistration Eligibility Decision (RED) and it was determined that 2,4-D was eligible for reregistration and the final notice not to initiate Special Review was issued (EPA 2005a).

The total annual use of 2,4-D in the United States was approximately 46 million pounds, based on data collected from 1992 through 2000. Agricultural use accounted for 66%, or 30 million pounds, while non-agricultural use accounted for 34%, or 16 million pounds. Broken down into area of use in terms of pounds, total 2,4-D use was distributed in the following pattern: pasture and rangeland, 24%; residential lawn with fertilizer, 12%; spring wheat, 8%; winter wheat, 7%; lawn and garden by lawn care and landscape professionals, 7%; residential lawn without fertilizer, 6%; field corn, 6%; soybeans, 4%, summer fallow, 3%; hay not including alfalfa, 3%, and roadways, 3% (EPA 2005a). Use varies from year to year. The U.S. Geological Survey (USGS) Pesticide National Synthesis Project estimated that approximately 38 million pounds of 2,4-D was applied to crops in 2014, with pasture and hay fields, wheat, soybeans, and corn crops receiving the greatest applications (USGS 2016). The development of genetically modified crops that have an increased tolerance to 2,4-D may cause an increase in the total amount applied annually to crops such as soybeans (EPA 2016a). Recently, the EPA granted the registration of a new herbicide named Enlist Duo™ containing 2,4-D choline salt and glyphosate for use on genetically modified corn, soybean, and cotton crops designed to be resistant to 2,4-D and glyphosate (EPA 2017).

5.2.4 Disposal

2,4-D should be disposed of by means in accordance with local regulations, such as incineration (Meister et al. 2014).

2,4-D is known to be degraded by soil microorganisms, and therefore, burial in non-crop areas away from water supplies may be an acceptable method of disposal for small quantities (HSDB 2015). The most environmentally acceptable means for 2,4-D disposal is by incineration. Triple rinsing and draining are used for the decontamination of 2,4-D containers and drums. Chemical treatment involves detoxification with chloride of lime or sodium carbonate. Removal of 2,4-D from water may be achieved through the use of activated charcoal or by coagulation and complete treatment by ozonation (HSDB 2015).

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005b). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time

employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005b).

5.3.1 Air

Estimated releases of 1,163 pounds (~0.53 metric tons) of 2,4-D to the atmosphere from 27 domestic manufacturing and processing facilities in 2016, accounted for about 0.34% of the estimated total environmental releases from facilities required to report to the TRI (TRI16 2018). These releases are summarized in Table 5-3.

2,4-D is released to the air during application with a wide range of equipment including fixed-wing aircraft, backpack sprayer, band sprayer, boom sprayer, ground directed sprayer, handheld sprayer, helicopter, and tractor-mounted sprayer as well as airblast and chemigation application (EPA 2005a). Available information on the releases of 2,4-D to the air in occupational settings and indoor air, along with exposure levels, is provided in Section 5.6.

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use 2,4-D[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AR	1	1	0	0	0	0	1	No data	1
CA	1	2	0	0	15	0	2	15	17
GA	1	0	0	0	0	481	0	481	481
IA	2	481	0	0	0	0	481	No data	481
IL	3	14	0	0	1,073	0	14	1,073	1,087

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use 2,4-D[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
IN	1	0	No data	0	0	0	No data	No data	No data
KS	2	10	0	0	188	0	10	188	198
MI	2	144	17	0	277,711	0	277,872	0	277,872
MO	1	204	0	0	669	0	204	669	873
MS	1	0	0	0	0	0	0	No data	0
MT	1	10	0	0	0	0	10	No data	10
NE	1	10	0	0	750	0	10	750	760
OH	4	256	0	56,898	13	135	57,154	148	57,302
PA	1	0	No data	0	0	0	No data	No data	No data
TN	1	2	0	0	0	0	2	No data	2
TX	2	28	0	0	0	0	28	No data	28
UT	1	2	0	0	0	0	2	No data	2
WI	1	0	No data	0	0	0	No data	No data	No data
Total	27	1,163	17	56,898	280,419	616	335,789	3,324	339,113

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, wastewater treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI16 2018 (Data are from 2016)

5.3.2 Water

Estimated releases of 17 pounds (~0.01 metric tons) of 2,4-D to surface water from 27 domestic manufacturing and processing facilities in 2016, accounted for <0.01% of the estimated total environmental releases from facilities required to report to the TRI (TRI16 2018). This estimate includes

releases to wastewater treatment and publicly owned treatment works (POTWs). These releases are summarized in Table 5-3.

2,4-D may enter the aquatic environment through direct application to water for weed control, disposal of wastes from manufacturing and production plants, runoff from treated lands, and drift from application (Sikka et al. 1976).

In 1969, a monitoring program of the irrigation water in the Columbia Basin in Washington reported that the 2,4-D application rate on canal bank weeds ranged from 1.4 to 2.5 pounds per acre (lbs/A) (1.57–2.8 kg/hectare) for a distance of up to 5.1 miles (Bartley and Hattrup 1970). During April–June 1969, about 170,000 gallons of 2,4-D (dimethyl amine salt) was applied to over 18,000 surface acres of Nickajack and Guntersville Reservoirs in Tennessee (Wojtalik et al. 1971). 2,4-D is used to treat aquatic waterbodies for the invasive European water chestnut (*Trapa natans L.)* and Eurasian water milfoil; this likely accounts for most of the intentional releases of this substance to surface waters. For example, in the summers of 2006, 2007, and 2008, 2,4-D was applied at a rate of 150, 200, and 200 lbs/A, respectively, to a 40-acre wetland in Oneonta, New York in close proximity to the Susquehanna River in order to eradicate overgrowth of water chestnut in this water body (Eyres 2009). 2,4-D formulations (Navigate®, Aquacide® and AquaKleen®) were also applied to a lake in East Haddam, Connecticut between 1999 and 2001 to control milfoil (Bugbee et al. 2003). Most states have strict use guidelines on using 2,4-D in aquatic environments and may require the use of a permit from the state's department of environmental conservation in order to apply these formulations to water bodies. The maximum 2,4-D (acid equivalent) rate for aquatic uses on submerged aquatic plants set by the EPA is 10.8 pounds/acre foot (EPA 2005a).

Effluent samples collected from 52 of the largest municipal wastewater treatment plants and water pollution control facilities in Oregon contained 2,4-D in 3 of 102 samples at a median concentration of 1,630 ng/L and a maximum concentration of 1,890 ng/L in 2010 (Hope et al. 2012).

5.3.3 Soil

Estimated releases of 280,419 pounds (~127.19 metric tons) of 2,4-D to soil from 27 domestic manufacturing and processing facilities in 2016, accounted for about 82.69% of the estimated total environmental releases from facilities required to report to the TRI (TRI16 2018). An additional

56,898 pounds (~25.81 metric tons), constituting about 16.78% of the total environmental emissions, were released via underground injection (TRI16 2018). These releases are summarized in Table 5-3.

More than 3.8 million kg (8.4 million pounds) of 2,4-D were applied to cereal crops in the three prairie provinces (Alberta, Saskatchewan, and Manitoba) of Canada in 1990 (Waite et al. 2002).

The rate per application and rate per year for 2,4-D (acid equivalent) are typically <1.5 and 2.0 pounds/acre/year, respectively (EPA 2005a). The maximum rate for asparagus, forestry uses, and non-cropland uses is 4.0 pounds/acre/year.

Because of its rapid biodegradation in soil, 2,4-D is not likely to be found in soil, except possibly near point sources after immediate release.

5.4 ENVIRONMENTAL FATE

The dominant process affecting the overall environmental fate of 2,4-D is degradation by microbiological activity (Wilson et al. 1997).

5.4.1 Transport and Partitioning

Air. Based on the vapor pressure of 2,4-D (see Table 4-3), 2,4-D released to the atmosphere via spraying applications would be expected to exist in both the vapor and particulate phases (Bidleman 1988).

Water. 2,4-D is released to water both from direct application for weed control, and through unintentional processes such as spray drift and runoff. Volatilization is not expected to be significant from water since most formulations of 2,4-D are as salts, which do not volatilize. 2,4-D released to water is not expected to be adsorbed to soils and sediments based on its organic carbon partition coefficient (Koc) values (EPA 1980, 2005; Rao and Davidson 1982; USDA 2001).

Sediment and Soil. 2,4-D released to soil partitions to surface water via runoff and to groundwater as a result of leaching. Volatilization of 2,4-D from moist and dry soils is not expected to be a significant transport process. 2,4-D ethylhexyl ester (2,4-D EHE) applied to a sandy loam at a rate of 15.8 lbs/acre was not volatile (<0.22% of the initial amount volatilized) over the course of a 30-day experiment (EPA

2004). It was observed that 2,4-D EHE rapidly transformed to 2,4-D (half-life 8 days), which is expected to exist as an anion under environmental conditions, and anions do not volatilize.

The mobility of 2,4-D in soils and sediments is expected to be high based on measured organic carbon corrected soil adsorption coefficient (K_{oc}) values. An average K_{oc} value of 19.6 was reported in nine soils tested (Rao and Davidson 1982). EPA (1980) measured an average K_{oc} of 109.1 in three soils (a silty clay loam, a sandy clay loam, and fine sand) with a range of 72.2–135.7. This study also reported that as the concentration of 2,4-D in the soil solution phase increased, the mobility increased. The ARS Pesticide Property Database lists Koc values for 2,4-D ranging from 20 to 79 (USDA 2001). Koc values of 70, 76, 59, and 117 were measured using a sandy loam, sand, silty clay loam, and loam soil, respectively (EPA 2005a). Despite the relatively low soil adsorption coefficients of 2,4-D, field dissipation studies have typically indicated only moderate leaching to lower soil levels due to the relatively rapid rate of degradation of 2,4-D (EPA 2004, 2005; Wilson et al. 1997).

2,4-D usually exists as an anion in the environment based its pKa of 2.73 (USDA 2001). Anionic compounds generally adsorb less than their neutral forms to clay or soils with organic carbon (Doucette 2000). Vasudevan and Cooper (2004) showed that soil mineralogy (iron and aluminum oxide content) and exchangeable aluminum content had a direct relationship with the adsorption of anionic 2,4-D, while soil phosphate content had an inverse effect, suggesting that 2,4-D will be more easily leached in soils subject to continued phosphate fertilization and liming. Soil pH also has an effect on mobility. In a study of four soils from rice-producing areas of Arkansas at pH 5 and 7, the mean adsorption coefficient (Kd) of 2,4-D ranged from 0.06 to 0.59 L/kg, and demonstrated that sorption was greatest and mobility was lowest at lower pH, as more of the substance would exist as the fully protonated acid rather than the conjugate base (Johnson et al. 1995).

Other Media. Bioaccumulation in aquatic organisms is not expected to be significant, based on a measured bioconcentration factor (BCF) of one for carp (Cyprinus carpio) exposed to 1 mg/L of 2,4-D for 28 days (NITE 2010a). Daphnid (Daphnia magna, a sand flea) and channel catfish (Ictalurus melas) exposed to 0.01 ppm 2,4-D over a period of 4 days had measured depuration half-lives of 13.8 hours and 1.32 days, respectively (Ellgehausen et al. 1980). Rodgers and Stalling (1972) performed a study in which fed and fasted bluegills and channel catfish were exposed to 1.0 mg/L of 14C-labeled 2,4-D butoxyethanol ester for up to 120 hours. Fed channel catfish and bluegills contained 7.3 and 7.8 µg/g (whole body) of 2,4-D after 1 hour of exposure. These levels decreased to 0.04 and 0.45 µg/g, respectively, after 24 hours of exposure, suggesting that uptake and elimination are rapid, but the rates are

different for the two species of fish. Similar trends were observed in the fasted fish. Whole-body levels of 9.03 and 16.67 µg/g of 2,4-D after 1 hour of exposure were observed for catfish and bluegills, respectively. These levels increased to 15.74 and 54.55 µg/g, respectively, after 6 hours and then declined to 1.20 and 7.50 µg/g, respectively, after 24 hours, indicating differential uptake and elimination rates between the species of fish. The slower elimination rate of 2,4-D in bluegills versus the channel catfish was further evidenced by the examination of 2,4-D residues in certain organisms of the fish. For example, blood samples of bluegills contained 20.9 µg/g after 8 hours of exposure, whereas catfish contained only 0.1 µg/g; liver samples of catfish contained 0.5 µg/g, while liver samples of bluegills contained 37.6 µg/g after 8 hours.

Bioaccumulation factors of 6 and <10 were reported for exposure to 50 µg/L 2,4-D in algae after 24 hours in a static system and in golden orfe (a fish) after 3 days, respectively (Freitag et al. 1982). Three seaweed species, Ulva sp., Enteromorpha sp., and Rhodymenia sp., exposed to 25 ppb of 2,4-D had a measured uptake of 0.01–0.03% after 24 hours of exposure (Sikka et al. 1976).

5.4.2 Transformation and Degradation

Degradation of 2,4-D is primarily by microbiological activity (Wilson et al. 1997). 2,4-D has been shown to undergo degradation in pure cultures by particular species of aerobic microorganisms. The two main pathways of degradation break apart bonds and transform the molecule, creating a hydroxyphenoxy acetic acid intermediate or by acting upon the corresponding phenol (WHO 1989). Half-lives for 2,4-D range from 1.8 to 3.1 days via degradation with a mixture of activated sludge, soil, and sediment microorganisms (Liu et al. 1981).

Air. A structure estimation method (Meylan and Howard 1993) was used to approximate a 19-hour half-life for the reaction of 2,4-D with hydroxyl radicals based on a vapor phase reaction rate constant of $6.6x10^{-12}$ cm^3/molecule-second at 25°C. 2,4-D may be susceptible to photolysis by direct sunlight, based on an ultraviolet maxima in the 280–290 range for phenoxy herbicides in aqueous media (HSDB 2015).

Water. 2,4-D, present at 100 mg/L, reached 0% of its theoretical biological oxygen demand (BOD) in 4 weeks using an activated sludge inoculum at 30 mg/L in the Japanese Ministry of International Trade and Industry (MITI) test (NITE 2010b). However, in other studies, 2,4-D was shown to degrade significantly in sewage sludge. More than 90% of 2,4-D at a concentration of 10–100 ng was mineralized in sewage after 28 days (Subba-Rao et al. 1982). Rosenberg and Alexander (1980) reported that nearly all 2,4-D

applied to municipal sewage was degraded after 7 days, and that further additions of 2,4-D were degraded without a lag period.

Radiolabeled 2,4-D at an initial concentration of 4.63 µg/g had a first-order degradation half-life of 15 days using a sediment and water mesocosm maintained under aerobic conditions (EPA 2004). Soluble degradation products identified in the study were chlorohydroquinone and 2,4-dichlorophenol (DCP).

Nesbitt and Watson (1980) showed that the rate of degradation of 2,4-D in river water was directly related to the sediment load and the nutrient concentration; however, the addition of organisms capable of degradation had no effect. 2,4-D incubated in sediment and unfiltered river water obtained during flood conditions degraded quickly with and without the addition of nutrients, which suggests that the water already possessed high phosphorous and nitrogen levels capable of sustaining microbial populations that degrade 2,4-D. This study reported ranges of half-lives of 2,4-D in river water from 18 to >50 days for clear water with low nutrient loadings and from 10 to 25 days for muddy (nutrient and sediment rich) water obtained after heavy rainfall and flooding conditions with lag times of 6–12 days.

In natural lake water, the extent of mineralization of 2,4-D was reported as 72% in 50 days and was shown to be enhanced by levels of both organics (62.7–95.8% mineralization) and inorganics (84% mineralization) in the water (Wang et al. 1984). Mineralization was also shown to be more rapid at higher concentrations of 2,4-D. This was demonstrated in another study that reported 75–90% mineralization of 2,4-D at concentrations of ≤500 pg/mL in eutrophic lake water in 28 days, but 34% was mineralized at a concentration of 4.9 ng/mL (Subba-Rao et al. 1982).

Preconditioning of organisms to 2,4-D may also increase the rate of degradation. This was shown in a study of the biodegradation of 2,4-D in river water during seasonal variation, which indicated that during different seasons, there was an effect on both 2,4-D concentrations in the water and its degrading capacity (Watson 1977). In these experiments, river water and mud were collected throughout the year from rivers draining from an agricultural region with 2,4-D use and compared to samples collected from rivers draining from forest regions with no recorded 2,4-D use or fertilizer applications. Greater degradation of 2,4-D was observed in the river waters and muds from the agricultural region as compared to the forest region. This was most notable using samples collected after heavy rainfall and flooding conditions where nutrient loadings from fertilizer usage in the agricultural location was common in the runoff into the river. In addition, the soils and waters surrounding the agricultural area with a history of 2,4-D usage is likely to contain greater colonies of microorganisms acclimated to degrading 2,4-D and other herbicides as

compared to soils and water from the forest region with no history of herbicide usage. Other factors such as, but not limited to, nutrient load, amount of 2,4-D degrading bacteria, and rainfall amounts are also instrumental in how quickly and how much 2,4-D can be degraded.

2,4-D is stable to hydrolysis (EPA 2005a). In sodium phosphate-buffered waters at pH 2, 7, and 10, there was no observed hydrolysis of 2,4-D, present at 25 µg/L (Chamberlain et al. 2012). Radiolabeled 2,4-D EHE at an initial concentration of 30 µg/L had a first-order half-life of 99.7 days in pH 5 buffer solution, 48.3 days in pH 7 buffer solution, and 52.2 hours in pH 9 buffer solution (EPA 2004).

2,4-D may undergo some degree of photodegradation in surface waters. In a water solution irradiated at 356 nm, 2,4-D had reported photolysis half-lives of 2–4 days (Baur and Bovey 1974). 2,4-D had a half-life of 50 minutes in water irradiated at 254 nm with reaction products 2,4-dichlorophenol, 4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and polymeric humic acids (Crosby and Tutass 1966). Furman et al. (2013) studied the photolysis rate of 2,4-D and atrazine in surface water samples collected from agricultural areas in four drainages of the Columbia River Basin in Washington State. They attempted to correlate the photolysis rates with three water quality parameters: nitrate levels in the surface water, dissolved organic carbon levels, and amount of suspended solids in the water samples. An average photolysis rate constant of 0.039/hour was reported for 2,4-D in surface water samples irradiated using a xenon arc lamp, which corresponds to a photolysis half-life of about 18 hours (Furman et al. 2013). Photolysis rates were increased in waters with high nitrate levels as the irradiation of nitrate in surface waters results in the production of hydroxyl radicals, which oxidize 2,4-D and other organic substances. Levels of dissolved organic carbon also showed a positive correlation with the photolysis rate of 2,4-D; however, the levels of suspended solids were inversely proportional to the photolysis rate in the surface water samples at one location. Radiolabeled 2,4-D EHE had a first-order half-life of 128.2 days in pH 5 buffer solution when irradiated with natural sunlight, while a dark control had a half-life of 252.5 days in the pH 5 buffer (EPA 2004). The main photodegradation products were 2,4-D and 2,4-DCP (Furman et al. 2013).

Sediment and Soil. 2,4-D undergoes biodegradation in soils under most conditions and is not considered persistent. The rate of degradation is affected by nutrient levels, oxygen levels, moisture, temperature, presence of microorganisms, concentration of 2,4-D and whether the soils had previously been acclimated with 2,4-D or other similar herbicides (WHO 1989). Under differing conditions, typical reported half-lives of 2,4-D ranged from <1 day to several weeks (Eder and Weber 1980; Foster and McKercher 1973; Liu et al. 1981; Ou 1984; Rao and Davidson 1982). The EPA Registration Eligibility

Decision document for 2,4-D reported that its half-life in an aerobic mineral soil was 6.2 days with several noted metabolites, including 1,2,4-benzenetriol, 2,4-DCP, 2,4-dichloroanisole (DCA), and 4-chlorophenol (EPA 2005a).

Increased moisture, temperature, and organic matter stimulate the degradation of 2,4-D, as demonstrated in a study of the herbicide in two soil types under dry and moist conditions and at two different temperatures (Ou 1984). 2,4,-D was rapidly mineralized using surface soil samples (0–15 cm depth) of a Cecil loamy sand (pH 5.6, 0.9% organic carbon, 6% clay) and a Webster sandy loam (pH 7.3, 3.9% organic carbon, 25% clay) at four different soil moisture levels over a 31-day incubation period and an initial loading rate of about 10 µg 2,4-D per gram of soil (Ou 1984). The half-life of 2,4-D ranged from 3.9 to 9.4 days in the loamy sand and from 7.0 to 253.9 days in the sandy loam depending upon the water content of the soil at an incubation temperature of 25°C. The greatest degradation rates of 2,4-D occurred for both soils under moist conditions as opposed to dry conditions, suggesting that greater microbial activity occurred in moist as opposed to dry soils and that greater moisture content decreased the amount of bound residues in the soils.

Thirty field dissipation studies conducted in seven states using bare soils and four cropping practices over the 2-year period of 1993–1994 were used to assess the environmental fate of 2,4-D following its application as 2,4-D dimethyl amine salt and 2,4-D EHE with both liquid and granular applications (Wilson et al. 1997). The first set of studies used wheat and turf fields located in Colorado and North Carolina and pastures in Texas. The second set of studies used cornfields from Nebraska and Ohio, wheat fields from North Dakota, and pasture, bare soil and turf fields located in California. Soil half-lives ranged from 1.7 days for turf applications in North Carolina to 12.8 days to pasture fields in Texas during the first set of trials conducted in 1993 in which all applications of 2,4-D were applied as sprays. Half-lives ranged from 2.1 days (bare soil California) to 31.2 days (pasture North Dakota) in the second set of trials conducted in 1994 in which 2,4-D was applied as sprays. Slightly greater half-life ranges were observed for the granular applications as opposed to the liquid sprays, which may be due to the time required to release the herbicides into the soil matrix. Across these studies, <5% of applied 2,4-D leached further than 15 cm from the surface. Moisture content played a major role on the half-life, with higher moisture levels resulting in faster degradation. Since these compounds, and other commercial forms of 2,4-D, are converted rapidly in soil to the same anionic form, these studies were representative of 2,4-D and showed that the chemical form had little effect on the rate of dissipation.

The EPA performed an analysis of the half-life of 2,4-D in various soils depending upon whether it was applied in granular form or as a liquid concentrate (EPA 2004). The granular half-lives ranged from 5.1 to 24.6 days, with a median half-life of 11.9 days, while the concentrate form had half-lives ranging from 1.1 to 42.5 days, with a median half-life of 5.5 days (EPA 2004).

2,4-D EHE was broadcast applied as a spray at a nominal concentration of 4 lbs/acre to a forested sandy clay loam soil located in Georgia (EPA 2004). 2,4-D EHE transformed to 2,4-D, with half-lives of 1.7, 7.2, and 51 days in the protected soil (soil under the forest canopy), foliage, and leaf litter, respectively. 2,4-D EHE was only detected 2 times in exposed soil (not protected by the forest canopy) and was not detected in the exposed soil after 3 days. The half-life of the corresponding 2,4-D was 4 days in the exposed soil, 3.6 days in the protected soil, 23.5 days in foliage, and 52.2 days in the leaf litter (EPA 2004).

2,4-D is generally considered a nonpersistent herbicide; however, at very high application rates, it may be toxic to the microorganisms of some soils or require a prolonged lag period before degradation begins. In a study of 2,4-D applied to various soils representative of the major soil orders of the United States, the lag period and overall degradation rate were directly related to the application rate of 2,4-D (EPA 1980). Formulated and technical-grade 2,4-D degradation, as measured by CO_2 evolution, began around day 10 following applications of 2,4-D at 50 and 500 mg/kg; however, the lag period increased to approximately 21 days at an initial application of 5,000 mg/kg and 50 days at an application rate of 20,000 mg/kg using a Webster silty clay loam soil (EPA 1980). Almost no CO2 evolution was observed from a sandy loam over the 80-day incubation period at application rates of 5,000 and 20,000 mg/kg, and even the addition of nutrients to the soil did not stimulate degradation.

Preconditioning of organisms to 2,4-D may also increase the rate of degradation in soil. Rosenberg and Alexander (1980) reported 2,4-D added to soil inocula showed 90% degradation after 14 days, after which subsequent additions of 2,4-D was reduced by 70% after 3–4 days. In a long-term field experiment where 2,4-D was applied annually, the complete degradation time was reduced from 10 weeks after one application to 4 weeks after 19 years of annual application (Torstensson et al. 1975).

In a study of the degradation of 2,4-D in soils at different pH levels, the half-life of 2,4-D was 5–8 days in soils in the pH range of 5.0–8.5. Degradation was slower in acidic soils, with half-lives of 21 and 41 days in soils with pH 4.5 and 4.0, respectively (Torstensson 1978).

The half-life of 2,4-D applied to a sterilized soil at 4.31 µg/g and irradiated with sunlight was 68 days (EPA 2004).

Other Media. In a study of the degradation of 2,4-D in forest leaf litter from red alder, ceanothus, vine maple, bigleaf maple, or Douglas fir collected in western Oregon, 2,4-D was shown to degrade approximately 25–40% after 15 days (Norris and Greiner 1967).

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to 2,4-D depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of 2,4-D in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on 2,4-D levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-4 shows the lowest limit of detections that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-5.

Table 5-4. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air	0.015 mg/m^3	NIOSH 1994
Drinking water	0.078 ppb	EPA 1995a
Surface water and groundwater	0.078 ppb	EPA 1995a
Soil	0.11 µg/kg soil	EPA 1996b
Sediment	0.01 ppb (suspended sediment)	USGS 1987
Urine	0.05 ppb	Draper 1982

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-5. Summary of Environmental Levels of 2,4-D

Media	Low	High	For more information
Outdoor air (ppbv)	0.0017	4	Section 5.5.1
Indoor air (ppbv)	No reliable data	No reliable data	Section 5.5.1
Surface water (ppb)	0.003	37	Section 5.5.2
Ground water (ppb)	0.33	50	Section 5.5.2
Drinking water (ppb)	0.0011	58	Section 5.5.2
Food (ppb)	0.03	1.69	Section 5.5.4
Soil (ppb)	8	143	Section 5.5.3

Detections of 2,4-D in air, water, and soil at NPL sites are summarized in Table 5-6.

Table 5-6. 2,4-D Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (ppb)	575	412	39.6	12	9
Soil (ppb)	2,750	2,660	3.26	10	6
Air (ppbv)			No data		

[a]Concentrations found in ATSDR site documents from 1981 to 2019 for 1,854 NPL sites (ATSDR 2019). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

Levels of 2,4-D in the ambient atmosphere are generally low or below the detection limits of the analytical methods used to monitor for this substance, with the exception of areas where it is applied as an herbicide and may reach levels in the low µg/m^3 range. In areas of high use of 2,4-D in Canada, such as cultivated regions, about 40% of air samples collected contained between 0.01 and 0.1 µg/m^3 (WHO 2003). In a monitoring study of the air quality in citrus growing regions of the United States, only 1 of 880 air samples contained 2,4-D at a concentration of 4 µg/m^3 (WHO 2003).

In a study that sampled air from nine locations, both urban and rural, in the United States in 1967 and 1968, 2,4-D was detected in one urban sample in Salt Lake City, Utah at a maximum concentration of 4.0 ng/m^3 (Stanley et al. 1971). 2,4-D was not detected in the air of any of the rural areas sampled, which

included locations outside of Buffalo, New York; Dothan, Alabama; Iowa City, Iowa; Orlando, Florida, and Stoneville, Mississippi. During the spraying season in Saskatchewan, Canada in 1972, the 33-day mean daily air concentrations of 2,4-D in urban Saskatoon was 600 ng/m^3, and the 47-day mean daily level was 142 ng/m^3 in Naicam (Que Hee et al. 1975).

In air samples collected in rural south-central Washington at seven and eight stations in 1973 and 1974, respectively, the average 2,4-D concentrations detected were 0.31 and 0.22 µg/m^3, respectively (Farwell et al. 1976). It was reported that the source of 2,4-D was from spray drift from nearby croplands.

In a study of 2,4-D atmospheric levels in an agricultural location in Saskatchewan, Canada where this herbicide was used extensively to treat weed infestations in cereal crops, 2,4-D was detected in 44–63% of the atmospheric samples obtained in the summer of 1989 and 33–53% of the samples obtained in the summer of 1990 (Waite et al. 2002). Mean concentrations ranged from 0.21 to 0.77 ng/m^3 in 1989 and from 0.17 to 0.49 ng/m^3 in 1990. The maximum air concentration of 2,4-D in samples in the summers of 1989 and 1990 was 3.90 ng/m^3 (Waite et al. 2002). 2,4-D detections in 1989 were attributed to atmospheric transport of wind-eroded soils from treated fields in nearby locations since this herbicide had not been applied near the sampling sites in that summer. The authors also studied the bulk atmospheric deposition of 2,4-D for both of the summers and noted that the highest deposition rates occurred during the month of June, which was the time that the majority of 2,4-D was applied in the region. The maximum bulk deposition rate was 3,550 ng/m^2-day in the summer of 1989 and 1,550 ng/m^2-day in the summer of 1990 (Waite et al. 2002).

2,4-D was detected in indoor air in a study of 13 residences following application to lawn surfaces (Nishioka et al. 2001). No 2,4-D was detected in any indoor air samples 1 week prior to application; however, widespread contamination of both the indoor air and home surfaces (e.g., carpets, floors, etc.) was noted postapplication with notable differences in levels depending upon whether the application was performed by the homeowner or a commercial contractor. Within 2 hours of homeowner application, average 2,4-D levels were approximately 9 and 4 ng/m^3 for PM10 and PM2.5 associated particle sizes, respectively, and about 4 (PM10) and 1 (PM2.5) ng/m^3 following contractor application. By day 3 postapplication, the average levels had decreased to about 3 (PM10) and 1 (PM2.5) ng/m^3 in the residences treated by the homeowner and about 2 (PM10) and 1 (PM2.5) ng/m^3 in the residences treated by the contractors. The main route of contamination was reported to be track-in practices by the homeowners and their pets.

5.5.2 Water

The widespread use of 2,4-D can result in its occurrence in surface water, groundwater, and drinking water, with concentrations typically in the µg/L range (Botre et al. 2000; USGS 2007).

According to USGS National Water Quality Assessment Program (NAWQA), which monitors groundwater and surface water across the major watersheds in the United States, 2,4-D was one of the most common substances detected in surface water during the 1992–2001 sampling period (USGS 2007). It was detected in roughly 20% of all agricultural streams and 11% of urban streams studied, but was only infrequently detected in undeveloped and mixed land use streams (USGS 2007). Annual maximum concentrations of 2,4-D ranged from 0.003 to 15 µg/L in 4,377 surface water samples obtained from the NAWQA dataset (EPA 2005a).

Over 50% of surface water samples collected from Lakes Ontario, Erie, Huron, and Superior between 1994 and 2000 had detectable concentrations of 2,4-D, with the maximum concentration measured being 0.08 µg/L. The highest concentrations were found near agricultural and urban environments where 2,4-D is used, such as the western basin of Lake Erie (Klecka et al. 2010).

In a study of California surface waters conducted between 2008 and 2011 in three urban areas that included Sacramento (SAC), San Francisco Bay (SFB), and Orange County (OC), 2,4-D was detected in 80–84% of samples collected from SAC and OC, and 66% of samples from SFB (Ensminger et al. 2013). Median concentrations for 2,4-D in SAC, SFB, and OC were approximately 0.4, 0.2, and 0.3 µg/L, respectively. During rainstorm events and increased runoff, the detection frequency and concentration increased. Median concentrations of 2,4-D in the dry season and during a rainstorm were 0.08 and 0.28 µg/L, respectively.

One day after the application of 2,4-D to 7,000 acres in the Loxahatchee National Wildlife Refuge in Florida to control the invasive plant, water hyacinth, at a rate of 4.48 kg/hectare (3.99 lbs/A), the concentration of 2,4-D in surface water in the Hillsboro Canal was 37 µg/L, which decreased to 1–4 µg/L 56 days later (Schultz and Whitney 1974). Eight hours following application of 2,4-D at a rate of 40 lbs/A to the Nickajack and Guntersville Reservoirs in Tennessee to treat invasive Eurasian watermilfoil, levels of about 5,000 µg /L were observed at the water surface and concentrations of 1,500 µg /L were observed at the root depth (Wojtalik et al. 1971). At 2 weeks postapplication, the 2,4-D content was uniformly 650 µg /L and at 1 month postapplication, it was 1 µg /L. Surface water samples

collected 4–6 times annually from November 1991 to June 1995 in South Florida had a maximum 2,4-D concentration of 14 µg/L (three detections) (Miles and Pfeuffer 1997). In a study to determine the presence of pesticides in 12 surface water supply intakes in Piedmont and coastal plain regions of North Carolina that were sampled in 1995, 2,4-D was detected in 7% of samples at a concentration range of not detected to 2.42 µg/L (Holman et al. 2000).

From April to September 2007, urban river and stream samples were collected from 19 sites within 16 watersheds, including 15 sites downstream from urban lands, across Canada and analyzed for acidic herbicides (Glozier et al. 2012). 2,4-D concentrations ranged from about 0.010 to 0.60 µg/L. Increased concentrations downstream of urban centers were linked to urban use. In agricultural watersheds sampled in Ontario, Canada from 1981 to 1985, 2,4-D was detected in approximately 9, 6, and 30% of the water samples taken from the mouth of the Grand, Saugeen, and Thames river basins, respectively (Frank and Logan 1988). Mean concentrations of 2,4-D ranged from 0.01 to 0.3 µg/L in the Grand River, from 0.1 to 0.2 µg/L in the Saugeen River, and from 0.3 to 0.7 µg/L in the Thames River (Frank and Logan 1988).

In a 1990 Puget Sound Pesticide Reconnaissance Survey, 15 water samples were collected from five drainage areas that empty into the Puget Sound in Washington and were assessed for pesticide residues (EPA 1991b). 2,4-D was detected in 13 water samples at concentrations ranging from 0.077 to 0.70 µg/L.

Even though 2,4-D is expected to have high mobility in soil, it was detected in <1% of all of the groundwater wells studied from 1992 to 2001 in the NAWQA survey due to its low persistence (USGS 2007). During the NAWQA assessment from 1992 to 1996, in which 2,485 groundwater sites were sampled in 20 of the major hydrologic basins in the United States, 2,4-D was detected in 0.43% of samples, with a maximum concentration of 0.54 µg/L (Kolpin et al. 2000). At 36 U.S. golf courses sampled in 1996, 2,4-D was detected in 8 of 773 groundwater samples at a maximum concentration of 50 µg/L (Cohen et al. 1999). Maximum and mean 2,4-D concentrations of 49.5 and 1.2 µg/L, respectively, were detected in 5 of 50 groundwater samples during a national survey of pesticides in groundwater (EPA 1988).

In the National Contaminant Occurrence Database, 27 of 71 lake/reservoir stations sampled contained a mean dissolved 2,4-D concentration of 0.33 µg/L (range of 0.01–10 µg/L) (EPA 2015b). In 73 of 256 stations where other surface waters were sampled, dissolved 2,4-D was detected at a mean concentration of 0.36 µg/L (range of 0.01–15 µg/L). The mean dissolved 2,4-D in groundwater detected at 5 of 465 stations sampled was reported as 4.0 µg/L (range of 0.01–24 µg/L).

During a study of drinking water supplies in the northern Great Plains of Canada, 15 reservoirs were sampled for pesticides during a spring application period (May to August, 2003) (Donald et al. 2007). 2,4-D was detected in all 206 samples collected, with a maximum reported concentration of 1,850 ng/L (1.850 µg/L). Mean concentrations for reservoirs in Manitoba, Saskatchewan, and Alberta were 46–182, 27–254, and 12–597 ng/L (0.046–0.182, 0.027–0.254, and 0.012–0.597 µg/L), respectively. Atmospheric deposition, snowmelt, and runoff was suspected as the major environmental transport processes responsible for 2,4-D in the reservoirs. The U.S. Department of Agriculture (USDA) Pesticide Data Program (PDP) analyzed 14 groundwater samples from 14 different wells, which included 3 from school/childcare wells and 11 from private wells in 2013 (USDA 2014). 2,4-D was detected in one sample. Additionally, 2,4-D was detected in 49 of 50 finished drinking water samples at concentrations ranging from 1.1 to 84 ng/L (0.0011–0.084 µg/L) (USDA 2014). It was also detected in 49 of 50 unfinished drinking water samples at concentrations ranging from 1.1 to 99 ng/L (0.0011–0.099 µg/L). Data from the EPA National Contaminant Occurrence Database indicated that 2,4-D was identified at 60 of 415 public water systems derived from surface water sources and 52 of 3,029 public water systems derived from groundwater at mean levels of 1.18 µg/L (range of 0.1–58 µg/L) and 0.87 µg/L (range of 0.08–8 µg/L), respectively (EPA 2015b).

Rainwater collected between February and October 1996 in Gruze, Switzerland had median and maximum 2,4-D concentrations of 16 and 23 ng/L (0.016 and 0.023 µg/L), respectively (Bucheli et al. 1998).

5.5.3 Sediment and Soil

In soil samples collected from one uncultivated and one cultivated California vertisol soil, 2,4-D concentrations ranged from 8 to 143 ppb at the uncultivated site and was not detected at the cultivated site (Graham et al. 1992). In 13 agricultural soils sampled in Canada between 1987 and 1992, the concentration of 2,4-D ranged from not detected to 38 mg/kg dry weight (Webber and Wang 1995).

In sediment samples collected from Lakes Ontario, Erie, Huron, and Superior from 1994 to 2000, 2,4-D was detected in over 50% of the samples at maximum concentrations of 1.04, 0.74, 0.28, and 0.8 µg/L, respectively (Klecka et al. 2010). Sediment samples taken from the Detroit River and Lake Huron in 1978 contained detectable levels of 2,4-D; however, the concentrations weren’t quantified (Konasewich et al. 1978).

5.5.4 Other Media

During the FDA's Market Basket study that tested 234 ready-to-eat foods 37 times a year between 1982 and 1991, the 10-year average concentration of 2,4-D detected was 0.006 µg/g (Rogers et al. 1995). Levels of 2,4-D in domestic foodstuffs were determined as part of FDA's 2004–2005 Total Diet Studies series (FDA 2005). The food samples were collected between October 2003 and August 2005. 2,4-D was detected in 22 out of 96 food items analyzed for. Twenty-one out of 22 detections were reported at the detection limit of the analytical method. The mean concentrations in µg/g (ppm) reported for 2,4-D in food items were as follows: white, enriched rice, 0.00025; white bread, 0.00060; whole wheat bread, 0.00169; fruit-flavored sweetened cereal, 0.00001; shredded wheat cereal, 0.00012; raisin bran cereal, 0.00035; crisped rice cereal, 0.00006; oat ring cereal, 0.00010; turkey and rice baby food, 0.00004; cracked wheat bread, 0.00098; rice cereal baby food, 0.00003; and meatless, Chinese fried rice, 0.00015. The most frequent detections of 2,4-D were found in bread products (FDA 2005). In 1971, 2,4-D was detected in 7 of 4,638 samples of dairy milk (Duggan et al. 1983).

Following the application of 2,4-D to 7,000 acres in the Loxahatchee National Wildlife Refuge in Florida at a rate of 4.48 kg/hectare (3.99 lbs/A), 2,4-D was detected in the breast muscle and liver of Florida gallinules at concentrations of 0.30 and 0.675 mg/kg, respectively, 1 day after spraying. Four days after spraying, no 2,4-D was detected. In 60 fish sampled, 19 had detectable 2,4-D residues in muscle tissue at concentrations ranging from <0.010 to 0.162 mg/kg (Schultz and Whitney 1974).

After treatment of the Nickajack and Guntersville Reservoirs on the Tennessee River with 2,4-D in 1969, concentrations in plankton 1, 8, and 24 hours and 14, 28, 30, 120, and 160 days after application were 0.06, 0.88, 1.8, 2.6, 3.6, 2.2, 1.1, and 3.7 ppm, respectively (Wojtalik et al. 1971). Whole body concentrations of eight species of freshwater fish from the Guntersville Reservoir did not rise above the pretreatment level of <0.10 mg/kg, with the exception of gizzard shad which had concentrations of 0.34, <0.10, 0.22, and <0.10 mg/kg at 28, 60, 120, and 180 days after application, respectively.

5.6 GENERAL POPULATION EXPOSURE

The general population may be exposed to 2,4-D during and after its use in residential and recreational areas. These include application to residential lawns, golf courses, parks, cemeteries, wooded areas, and other grassy areas. Since 2,4-D is also used on aquatic weeds, swimmers may be exposed when

swimming in waters treated with 2,4-D (EPA 2005a). Transport of 2,4-D into residential homes may occur from agricultural spray drift, volatilization, soil or dust resuspension, tracked in on shoes, and on clothing (Nishioka et al. 2001). 2,4-D exposure for the general population is typically at or near the level of detection (CDC 2015). The reported limit of detection values ranged from 0.2 to 20 µg/L in the biomonitoring and epidemiology studies reviewed.

NHANES uses biomonitoring to provide estimates of exposure to the civilian U.S. population. Chemicals and their metabolites are measured in subsets of participants aged 6–59 years old, meant to be a representative sample of the population. Urine measurements are reported as both the concentration in urine and the concentration corrected for urine-creatinine level, which adjusts for urine dilution. Urinary levels of 2,4-D were measured in several NHANES programs assessing exposure to subsets of the general population in the United States from years 1999–2000, 2001–2002, and 2003–2004, 2005–2006, 2007–2008, and 2009–2010 (CDC 2015). For survey years 1999–2000, 2001–2002, and 2007–2008, no geometric mean urinary concentration of the 2,4-D could be calculated because the proportion of results below the detection limit was too high to provide a valid result. The NHANES results for 1999–2010 are summarized in Tables 5-7 and 5-8 (CDC 2015). Urinary levels have remained steady over the temporal period for the age and gender groups shown in the tables and represent a broad mix of the general public.

In a study of pesticide residues collected from 1,000 adults, ranging in age from 20 to 59 years, living in the United States, 2,4-D was detected in 12% of samples at a mean concentration of <1 µg/L (Hill et al. 1995). The 95th percentile and maximum concentrations were reported as 1.8 and 37 µg/L, respectively.

Table 5-7. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Total	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,977
	2001–2002	*	<LOD	0.220 (<LOD–0.310)	0.690 (0.560–0.880)	1.26 (1.01–1.36)	2,903
	2003–2004	0.245 (0.210–0.286)	0.230 (0.180–0.320)	0.580 (0.490–0.660)	1.10 (0.910–1.34)	1.71 (1.41–2.37)	2,488
	2007–2008	*	<LOD	0.550 (0.530–0.590)	1.06 (0.940–1.19)	1.60 (1.38–1.79)	2,587
	2009–2010	0.308 (0.275–0.345)	0.280 (0.250–0.320)	0.530 (0.470–0.600)	0.930 (0.810–1.08)	1.43 (1.12–2.02)	2,747

Table 5-7. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Age group							
6–11 years	1999–2000	*	<LOD	<LOD	<LOD	1.30 (<LOD–2.40)	477
	2001–2002	*	<LOD	0.310 (0.210–0.400)	0.740 (0.550–1.13)	1.55 (1.00–2.21)	546
	2003–2004	0.266 (0.214–0.332)	0.290 (0.200–0.390)	0.670 (0.440–0.920)	1.03 (0.890–1.40)	1.88 (1.01–2.54)	309
	2007–2008	*	<LOD	0.720 (0.630–0.860)	1.44 (1.15–1.64)	1.93 (1.62–2.84)	385
	2009–2010	0.385 (0.330–0.449)	0.350 (0.290–0.440)	0.670 (0.510–0.780)	1.20 (0.860–1.58)	1.59 (1.36–2.77)	386
12–19 years	1999–2000	*	<LOD	<LOD	<LOD	1.10 (<LOD–1.60)	677
	2001–2002	*	<LOD	0.250 (<LOD–0.420)	0.690 (0.440–1.16)	1.24 (.690–1.66)	797
	2003–2004	0.256 (0.212–0.310)	0.260 (0.180–0.380)	0.580 (0.470–0.710)	1.04 (0.890–1.31)	1.66 (1.20–2.97)	714
	2007–2008	*	<LOD	0.590 (0.530–0.670)	1.29 (0.790–1.97)	2.38 (1.46–2.73)	390
	2009–2010	0.301 (0.248–0.366)	0.280 (0.240–0.330)	0.490 (0.420–0.620)	0.900 (0.660–1.05)	1.12 (0.880–2.88)	401
20–59 years	1999–2000	*	<LOD	<LOD	<LOD	<LOD	823
	2001–2002	*	<LOD	0.210 (<LOD–0.310)	0.690 (0.540–0.910)	1.27 (0.930–1.49)	1,070
	2003–2004	0.239 (0.205–0.279)	0.220 (0.170–0.300)	0.570 (0.480–0.640)	0.980 (0.840–1.35)	1.55 (1.25–2.50)	937
	2007–2008	*	<LOD	0.530 (0.490–0.570)	0.970 (0.800–1.17)	1.36 (1.22–1.78)	1,179
	2009–2010	0.288 (0.259–0.319)	0.270 (0.230–0.310)	0.500 (0.440–0.560)	0.870 (0.740–1.04)	1.33 (1.05–1.69)	1,309
≥60 years	2001–2002	*	<LOD	<LOD	0.560 (0.390–0.870)	1.26 (0.690–1.78)	490
	2003–2004	0.248 (0.205–0.301)	0.210 (0.130–0.320)	0.560 (0.470–0.680)	1.36 (1.07–1.90)	2.42 (1.66–3.67)	528
	2007–2008	*	<LOD	0.560 (0.530–0.640)	1.02 (0.840–1.12)	1.46 (1.10–2.11)	633
	2009–2010	0.349 (0.294–0.414)	0.300 (0.230–0.390)	0.590 (0.510–0.720)	1.11 (0.810–1.57)	2.08 (1.16–5.40)	651

Table 5-7. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Gender							
Males	1999–2000	*	<LOD	<LOD	<LOD	1.10 (<LOD–1.80)	962
	2001–2002	*	<LOD	0.330 (0.220–0.490)	0.890 (0.690–1.17)	1.49 (1.26–2.03)	1,364
	2003–2004	0.276 (0.240–0.317)	0.290 (0.210–0.370)	0.630 (0.540–0.740)	1.22 (0.960–1.42)	2.12 (1.42–2.73)	1,218
	2007–2008	*	<LOD	0.610 (0.580–0.650)	1.26 (1.05–1.38)	2.11 (1.68–2.41)	1,292
	2009–2010	0.347 (0.298–0.404)	0.320 (0.270–0.370)	0.580 (0.500–0.690)	1.05 (0.810–1.47)	1.82 (1.12–4.14)	1,343
Females	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,015
	2001–2002	*	<LOD	<LOD	0.470 (0.360–0.620)	0.890 (0.670–1.21)	1,539
	2003–2004	0.219 (0.181–0.264)	0.190 (0.110–0.280)	0.490 (0.400–0.630)	0.980 (0.860–1.33)	1.48 (1.31–2.27)	1,270
	2007–2008	*	<LOD	0.500 (0.460–0.540)	0.870 (0.790–1.01)	1.28 (1.12–1.42)	1,295
	2009–2010	0.275 (0.250–0.303)	0.260 (0.220–0.300)	0.480 (0.440–0.540)	0.860 (0.740–0.950)	1.14 (.970–1.39)	1,404
Race/ethnicity							
Mexican Americans	1999–2000	*	<LOD	<LOD	<LOD	<LOD	695
	2001–2002	*	<LOD	0.250 (<LOD–0.330)	0.730 (0.610–0.890)	1.20 (.960–1.36)	743
	2003–2004	0.313 (0.256–0.383)	0.340 (0.260–0.440)	0.730 (0.610–0.840)	1.42 (1.02–1.52)	1.81 (1.23–3.53)	606
	2007–2008	*	<LOD	0.520 (0.470–0.590)	0.860 (0.790–1.00)	1.46 (0.950–2.22)	500
	2009–2010	0.276 (0.240–0.318)	0.250 (0.210–0.300)	0.470 (0.410–0.570)	0.840 (0.680–1.08)	1.23 (0.830–2.02)	602
Non-Hispanic blacks	1999–2000	*	<LOD	<LOD	<LOD	1.20 (<LOD–1.70)	520
	2001–2002	*	<LOD	<LOD	0.560 (0.420–0.890)	1.06 (0.790–1.48)	743
	2003–2004	*	0.190 (<LOD–0.290)	0.510 (0.380–0.630)	0.910 (0.750–1.22)	1.31 (0.990–1.98)	648
	2007–2008	*	<LOD	0.580 (0.530–0.630)	1.05 (0.910–1.20)	1.49 (1.23–1.97)	574
	2009–2010	0.284 (0.251–0.321)	0.260 (0.240–0.290)	0.460 (0.390–0.540)	0.790 (0.620–1.03)	1.11 (0.790–1.81)	504

Table 5-7. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Non-Hispanic whites	1999–2000	*	<LOD	<LOD	<LOD	<LOD	589
	2001–2002	*	<LOD	0.240 (<LOD–0.360)	0.730 (0.560–0.980)	1.30 (1.01–1.66)	1,201
	2003–2004	0.254 (0.211–0.306)	0.240 (0.180–0.360)	0.590 (0.470–0.720)	1.17 (0.930–1.41)	2.00 (1.40–2.51)	1,076
	2007–2008	*	<LOD	0.560 (0.540–0.600)	1.12 (0.940–1.29)	1.61 (1.36–2.16)	1,083
	2009–2010	0.328 (0.281–0.382)	0.300 (0.250–0.370)	0.570 (0.480–0.680)	0.980 (0.830–1.20)	1.57 (1.14–2.77)	1,200

CI = confidence interval

Source: CDC 2015

Table 5-8. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Total	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,977
	2001–2002	*	<LOD	0.378 (<LOD–0.412)	0.700 (0.635–0.778)	1.12 (1.03–1.26)	2,901
	2003–2004	0.241 (0.203–0.287)	0.253 (0.206–0.290)	0.500 (0.423–0.610)	1.03 (0.855–1.28)	1.85 (1.42–2.50)	2,486
	2007–2008	*	<LOD	0.737 (0.667–0.779)	1.28 (1.17–1.40)	1.84 (1.65–2.12)	2,585
	2009–2010	0.321 (0.286–0.360)	0.301 (0.272–0.329)	0.500 (0.458–0.573)	0.983 (0.846–1.19)	1.55 (1.30–2.12)	2,747
Age group							
6–11 years	1999–2000	*	<LOD	<LOD	<LOD	1.32 (<LOD–2.24)	477
	2001–2002	*	<LOD	0.485 (0.378–0.679)	1.13 (0.825–1.35)	1.41 (1.27–1.73)	546
	2003–2004	0.323 (0.249–0.421)	0.320 (0.250–0.440)	0.744 (0.500–1.06)	1.30 (0.990–2.55)	2.55 (1.23–5.16)	309
	2007–2008	*	<LOD	0.970 (0.817–1.24)	1.65 (1.47–1.85)	2.96 (1.65–6.18)	385
	2009–2010	0.521 (0.444–0.610)	0.478 (0.411–0.531)	0.792 (0.674–1.06)	1.52 (1.21–1.74)	2.20 (1.53–3.02)	386

Table 5-8. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
12–19 years	1999–2000	*	<LOD	<LOD	<LOD	0.593 (<LOD–1.05)	677
	2001–2002	*	<LOD	0.275 (<LOD–0.376)	0.483 (0.328–0.662)	0.662 (0.517–0.918)	796
	2003–2004	0.193 (0.160–0.232)	0.205 (0.157–0.250)	0.419 (0.328–0.460)	0.709 (0.540–0.925)	1.23 (0.837–2.35)	713
	2007–2008	*	<LOD	0.555 (0.475–0.651)	0.908 (0.778–1.05)	1.56 (0.950–2.79)	388
	2009–2010	0.258 (0.212–0.314)	0.256 (0.200–0.299)	0.358 (0.320–0.439)	0.706 (0.439–1.05)	1.05 (0.579–3.27)	401
20–59 years	1999–2000	*	<LOD	<LOD	<LOD	<LOD	823
	2001–2002	*	<LOD	0.378 (<LOD–0.412)	0.667 (0.593–0.778)	1.08 (0.806–1.29)	1,070
	2003–2004	0.227 (0.188–0.274)	0.242 (0.196–0.278)	0.452 (0.397–0.545)	0.923 (0.708–1.20)	1.48 (1.14–2.43)	936
	2007–2008	*	<LOD	0.667 (0.588–0.769)	1.17 (1.04–1.34)	1.65 (1.43–2.33)	1,179
	2009–2010	0.288 (0.259–0.321)	0.276 (0.250–0.309)	0.458 (0.418–0.507)	0.860 (0.750–0.962)	1.36 (1.00–1.88)	1,309
≥60 years	2001–2002	*	<LOD	<LOD	0.824 (0.583–1.10)	1.34 (1.00–2.16)	489
	2003–2004	0.301 (0.248–0.366)	0.310 (0.237–0.385)	0.657 (0.510–0.866)	1.54 (1.16–1.95)	3.00 (1.95–6.36)	528
	2007–2008	*	<LOD	0.860 (0.781–0.903)	1.53 (1.27–1.72)	1.96 (1.60–2.33)	633
	2009–2010	0.414 (0.356–0.480)	0.354 (0.306–0.407)	0.667 (0.548–0.812)	1.41 (0.983–1.99)	2.87 (1.49–4.49)	651

Table 5-8. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Gender							
Males	1999–2000	*	<LOD	<LOD	<LOD	0.667 (<LOD–1.16)	962
	2001–2002	*	<LOD	0.336 (0.272–0.412)	0.652 (0.560–0.825)	1.14 (0.979–1.39)	1,364
	2003–2004	0.227 (0.189–0.271)	0.238 (0.194–0.276)	0.473 (0.412–0.564)	0.941 (0.767–1.23)	1.80 (1.09–2.79)	1,217
	2007–2008	*	<LOD	0.596 (0.538–0.670)	1.14 (0.980–1.24)	1.63 (1.47–2.15)	1,291
	2009–2010	0.309 (0.266–0.359)	0.282 (0.242–0.323)	0.481 (0.413–0.554)	1.01 (0.707–1.57)	1.80 (1.06–3.88)	1,343
Females	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,015
	2001–2002	*	<LOD	<LOD	0.711 (0.631–0.809)	1.10 (0.933–1.26)	1,537
	2003–2004	0.256 (0.213–0.308)	0.263 (0.212–0.311)	0.522 (0.435–0.645)	1.14 (0.900–1.42)	1.85 (1.42–2.64)	1,269
	2007–2008	*	<LOD	0.854 (0.757–0.903)	1.47 (1.23–1.58)	1.91 (1.65–2.33)	1,294
	2009–2010	0.334 (0.302–0.369)	0.319 (0.288–0.355)	0.533 (0.475–0.611)	0.953 (0.862–1.10)	1.40 (1.21–1.55)	1,404
Race/ethnicity							
Mexican Americans	1999–2000	*	<LOD	<LOD	<LOD	<LOD	695
	2001–2002	*	<LOD	0.350 (<LOD–0.386)	0.720 (0.583–0.840)	1.08 (.778–1.56)	743
	2003–2004	0.287 (0.223–0.371)	0.309 (0.194–0.459)	0.593 (0.463–0.771)	1.08 (0.833–1.36)	1.54 (1.17–3.19)	605
	2007–2008	*	<LOD	0.622 (0.571–0.691)	1.15 (0.903–1.43)	1.74 (1.37–2.33)	499
	2009–2010	0.289 (0.255–0.326)	0.282 (0.255–0.300)	0.434 (0.392–0.495)	0.781 (0.565–1.11)	1.30 (.733–2.63)	602
Non-Hispanic blacks	1999–2000	*	<LOD	<LOD	<LOD	0.593 (<LOD–1.19)	520
	2001–2002	*	<LOD	<LOD	0.467 (0.349–0.583)	0.778 (0.552–0.975)	742
	2003–2004	*	0.140 (<LOD–0.194)	0.304 (0.264–0.356)	0.629 (0.461–0.815)	0.970 (0.719–1.50)	648
	2007–2008	*	<LOD	0.509 (0.457–0.596)	0.966 (0.875–1.07)	1.33 (1.12–1.75)	573
	2009–2010	0.215 (0.192–0.240)	0.195 (0.180–0.218)	0.344 (0.314–0.400)	0.628 (0.489–0.822)	1.06 (0.714–1.38)	504

Table 5-8. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Non-Hispanic whites	1999–2000	*	<LOD	<LOD	<LOD	<LOD	589
	2001–2002	*	<LOD	0.412 (<LOD–0.455)	0.769 (0.667–0.894)	1.25 (1.05–1.40)	1,200
	2003–2004	0.263 (0.213–0.326)	0.269 (0.226–0.318)	0.539 (0.434–0.665)	1.13 (0.941–1.46)	2.34 (1.54–2.73)	1,075
	2007–2008	*	<LOD	0.780 (0.737–0.871)	1.36 (1.17–1.55)	2.00 (1.60–2.49)	1,083
	2009–2010	0.357 (0.308–0.414)	0.328 (0.288–0.384)	0.547 (0.485–0.644)	1.10 (0.897–1.40)	1.79 (1.35–3.02)	1,200

CI = confidence interval

Source: CDC 2015

In the CTEPP study, the exposures of 135 preschool children and their adult caregivers to 2,4-D at their homes in North Carolina and Ohio were examined in 2000 and 2001 (Morgan et al. 2008). Monitoring was performed over a 48-hour period, and personal (hand wipes and food) and environmental (air, soil, and dust) samples were collected. 2,4-D was detected in all types of environmental samples, with the highest frequency in carpet dust samples at 83% (median concentration of 47.5 ng/g) and 98% (median concentration of 156 ng/g) in North Carolina and Ohio, respectively. Detection frequencies in North Carolina and Ohio were 38 and 49% (maximum concentrations of 3.7 and 2.0 ng/m^3) for indoor air, 19 and 34% (maximum concentrations of 1.7 and 3.2 ng/m^3) for outdoor air, and 17 and 45% (maximum concentrations of 30.5 and 13.3 ng/g) for soil, respectively. Maximum concentrations of 2,4-D in personal exposure samples for adults in North Carolina and Ohio were 0.02 and 0.1 ng/cm^2 for hand wipes and 4.0 and 3.7 ng/g for solid food, respectively. 2,4-D was detected in >85% of the total samples collected. The median 2,4-D urinary concentrations in adults were 0.7 ng/mL for both North Carolina and Ohio residents. Morgan (2015) examined urinary levels of 2,4-D and other pesticide biomarkers and compared sociodemographic and lifestyle factors with exposure levels. Geometric mean urinary levels of 2,4-D (0.80 ng/mL [µg/L]) in urine of younger adults aged 20–35 years were significantly higher ($p=0.0025$) when compared to levels (0.54 ng/mL [µg/L]) in older adults aged 36–49 years. The study also indicated that sweet/salty snack consumption, time spent outside the home, and creatinine levels were significant ($p<0.05$) predictors of urinary 2,4-D levels.

Indoor air, outdoor air, and urine samples were analyzed for 2,4-D in a study assessing the exposure of 20 home gardeners and 19 bystanders living within the household using the product. (Harris et al. 1992). The homeowners were divided into groups that wore protective and non-protective clothing and applied both a granular and liquid formulation. The protective apparel group applying liquid 2,4-D reported no 2,4-D in air samples collected outside and only one detection at 6.0 µg/m^3 in indoor air. The protective group using granular 2,4-D reported no 2,4-D in indoor samples and three detections in outdoor air, with a mean concentration of 2.9 µg/m^3. No 2,4-D was detected in the urine of bystanders in either protective group. Among the applicators, three had detections in urine at total concentrations of 108, 63, and 38 µg/person in 4 days, and these were all attributed to the applicator removing their gloves at some point during application. Analysis of urine samples collected from home gardeners 96 hours after application showed 2,4-D total body doses ranging from below detection to 0.0071 mg/kg of body weight. The total mean 2,4-D urine concentration of applicators using liquid and granular formulations were 203.6 and 18.8 µg/person in 4 days, respectively. Bystanders in both non-protective groups had no 2,4-D detections in urine. The highest exposures were found in the group wearing non-protective apparel and were associated with spills of the liquid formulation and dermal contact with the herbicide. There is a chance that bystanders could be exposed from treated turf grass immediately following application, although it has been shown that this may be <6% of the original amount of 2,4-D used.

Workers may be exposed to 2,4-D during mixing, loading, and applying, for both crop and non-agricultural uses (EPA 2005a). Families of workers may also be exposed to 2,4-D through home surfaces contaminated from contact with an applicator's hands or clothing. Deposition of 2,4-D contaminated dust or aerial dispersion from field spraying may also lead to surface contamination (Arbuckle et al. 2006).

In a biomonitoring study of exposure to 2,4-D in farm families with licensed applicators in Minnesota and South Carolina, 24-hour urine 2,4-D concentrations were collected 1 day before through 3 days after application (Alexander et al. 2007). For applicators (n=34), spouses (n=34), and children 4–17 years old (n=53), the median urine 2,4-D concentrations pre-application and 1 day after application were 2.1 and 73.1 µg/L, below the limit of detection and 1.2 µg/L, and 1.5 and 2.9 µg/L, respectively. At baseline, 2,4-D was detectable in the urine of 70% of the applicators, 41% of the spouses, and 62% of the children. The mean urine 2,4-D concentration in applicators and spouses the day before application, the day of application, 1 day after application, 2 days after application, and 3 days after application were 3.8 and 1.0, 29.1 and 1.0, 64.2 and 1.3, 45.3 and 1.4, and 28.3 and 1.3 µg/L, respectively. During and postapplication concentrations for applicators were substantially higher than baseline concentrations. Applicators who wore gloves to prevent direct skin contact had consistently lower urine 2,4-D concentrations, with the

mean concentration for applicators not wearing gloves >7 times greater (236 compared to 44 µg/L). Exposure to spouses was determined to be primarily attributable to the level of contact with the application process, including their presence during mixing or application of 2,4-D. The geometric mean urinary levels of 2,4-D in 69 herbicide applicators were 7.8 and 25 µg/L prior to 2,4-D application and 1 day following application, respectively (Thomas et al. 2010a, 2010b). The mean absorbed dose estimated for 14 2,4-D broadcast and spray applications was 0.0027±0.0044 mg/kg/day. The mean absorbed dose accounts from exposures from all sources, including application (dermal and inhalation) plus dietary ingestion and contact with 2,4-D containing surfaces in the home or farm.

In a study of repeated pesticide exposure to migrant and seasonal farmworkers in North Carolina, urine samples were collected from 196 farmworkers four times at monthly intervals in 2007 (Arcury et al. 2010). 2,4-D had at least one detection in 98% of farmworkers, and 86.7% had multiple detections.

While direct contact with 2,4-D during mixing, loading, application, or cleaning is the primary route of exposure for individuals living on a farm, indirect sources may also contribute. This includes contact with contaminated surfaces within the home (Arbuckle et al. 2006). In a biomonitoring study performed May through July 1996 to identify potential sources of 2,4-D exposure for families on farms, residues in drinking water and surface swipes of commonly touched surfaces with 32 Ontario farm homes were measured and compared to urinary concentrations found in applicators, spouses, and children. Surfaces tested were exterior door handles, refrigerator handles, kitchen faucet, washing machine knobs, bathroom faucet, wash-up faucet, telephone, toilet handle, and tractor steering wheel. 2,4-D was detected on all measured surfaces, with the highest levels found on the washing machine knob, wash-up faucet, and tractor steering wheel. For urine samples collected before application of 2,4-D, 66% of applicators, 44% of spouses, and 46% of children had a concentration ≥1 µg/L of 2,4-D, suggesting that 2,4-D used in previous seasons may be tracked indoors and persist on home surfaces. Mean concentrations of drinking water suggested that this is not an important route of exposure, as only 1% of homes had detectable levels of 2,4-D (Arbuckle et al. 2006).

A study was conducted measuring the levels of pesticides in urine and hand wipes among 24 farmer and 23 non-farmers in Iowa in the spring and summer of 2001 (Curwin et al. 2005a). Urine and hand wipe samples were collected from each person on two occasions, approximately 1 month apart. 2,4-D urinary concentrations were significantly higher in farmers who applied 2,4-D (mean of 13 µg/L), compared to farmers who had it commercially applied (mean of 1.6 µg/L), farmers who did not apply it (mean of 0.48 µg/L), and non-farmers (mean of 0.29 µg/L). It was shown that 2,4-D urine levels may be associated

with time since application, amount of 2,4-D applied, and number of acres to which it was applied. None of the 21 hand wipe samples collected had detectable 2,4-D residues. Urinary levels of 2,4-D were measured in corn farmers from Iowa over the period of March to November 2002 and 2003 (Bakke et al. 2009). Statistically significant increases in 2,4-D levels were observed during the planting season as compared to pre-planting and the offseason; however, differences remained significant even after the exclusion of urine samples obtained within 7 days of application, suggesting that exposure can continue well after application.

Curwin et al. (2005b) conducted a study of agricultural pesticide contamination in 25 farm homes and 25 nonfarm homes in Iowa by collecting air, surface wipe, and dust samples between May and August of 2001. Samples from 11 homes (5 farm homes and 6 nonfarm homes) were taken for 2,4-D detection. 2,4-D was found in 100% of farm and nonfarm dust samples, with concentrations of 0.0041–1.9 and 0.00099–5.3 ng/cm2, respectively. In farm and nonfarm homes, 2,4-D adjusted mean concentrations in dust were highest in the entryway, 850 and 740 ng/g, respectively, while in the child's bedroom, the mean concentrations were 660 and 450 ng/g, respectively. All outdoor air (n=98) and indoor air samples (n=99) were below the limit of detection. Of the 82 house wipe and 48 vehicle wipe samples, 2,4-D was below the detection limit for all samples. This study is another example that agriculturally used 2,4-D may be an important source of home contamination.

In workers spraying 2,4-D in wheat fields, concentrations detected in 165 urine samples from 34 workers ranged from 35 to 400 µg/L (Aprea et al. 1997).

A summary of urinary concentrations 2,4-D in workers is presented in Table 5-9.

Table 5-9. Measured 2,4-D Urine Concentrations for Workers

Occupation	Number of samples	Geometric mean (µg/L)	Notes	Reference
Farmer (applicator)	34	3.8, 29.1, 64.2, 45.3, and 28.3	Day before, day of, 1 day after, 2 days after, and 3 days after application, respectively	Alexander et al. 2007
Herbicide applicator	69	7.8 and 25	Prior to and 1 day after application, respectively	Thomas et al. 2010a, 2010b
Farmer (applicator)	48	13		Curwin et al. 2005a
Sprayers in wheat fields	165	35–400 (range)	34 workers sampled	Aprea et al. 1997

The National Occupational Exposure Survey (NOES) conducted by NIOSH in 1983 estimated that 471 workers employed at 94 facilities were potentially exposed to 2,4-D in the United States (RTECS 2009). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

A susceptible population will exhibit a different or enhanced response to 2,4-D than will most persons exposed to the same level of 2,4-D in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of 2,4-D, or compromised function of organs affected by 2,4-D.

No studies of populations unusually susceptible to 2,4-D toxicity were identified in the literature reviewed.

Studies in animals have shown that 2,4-D is eliminated from the body by active secretion into urine by means of an OAT1 carrier. This carrier protein, which is shared by many animal species including humans, was found to be developmentally-regulated in rats, as expression increased 4-fold between PND 5 and 35 in both male and female rats (Buist et al. 2002). If this were the case also in humans, neonates and/or infants could be at a higher risk for 2,4-D toxicity since lower renal clearance of 2,4-D has been associated with increased systemic toxicity of 2,4-D, as it occurs in dogs (Gorzinski et al. 1987).

A study in rats reported that undernourished pups were more vulnerable to the effects of 2,4-D (body weight, organ's weight) than well-nourished pups (Ferri et al. 2003). A later study from the same group of investigators confirmed the results regarding body weight and reported that undernourished pups also may be more vulnerable to the hypomyelinating effect of 2,4-D (Konjuh et al. 2008).

As discussed in Section 5.6, occupational exposure to workers during mixing, loading, and application of 2,4-D will likely result in higher-than-average exposures to this substance (EPA 2005a). The EPA RED outlines the Personal Protective Equipment (PPE) requirements for 2,4-D labeling for liquids, wettable powders, and water dispersible granules as well as pure granular formulations (EPA 2005a). In general, in order to reduce exposure, mixers, loaders, applicators flaggers, and other handlers should wear long-

sleeved shits/pants, shoes, and socks and chemical resistant gloves. Homeowners and their families who use 2,4-D for lawn treatment also have a higher potential for exposure than people who do not apply 2,4-D to their lawns. Homeowners applying 2,4-D should follow similar labeling procedures to reduce exposure. Families of workers may also be exposed through home surfaces contaminated from contact with an applicator's hands or clothing. In addition, families living proximal to treated fields, orchards, and managed forests/timber may have greater exposure than the general population.

Comparing urinary 2,4-D levels from the NHANES, 1999–2010, report to data from occupationally exposed workers indicates that urinary 2,4-D levels can be up to 100 times greater for workers shortly after application as compared to the general population in the 50th percentile (Alexander et al. 2007; CDC 2015; Thomas et al. 2010a, 2010b).

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2,4-D is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of 2,4-D.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

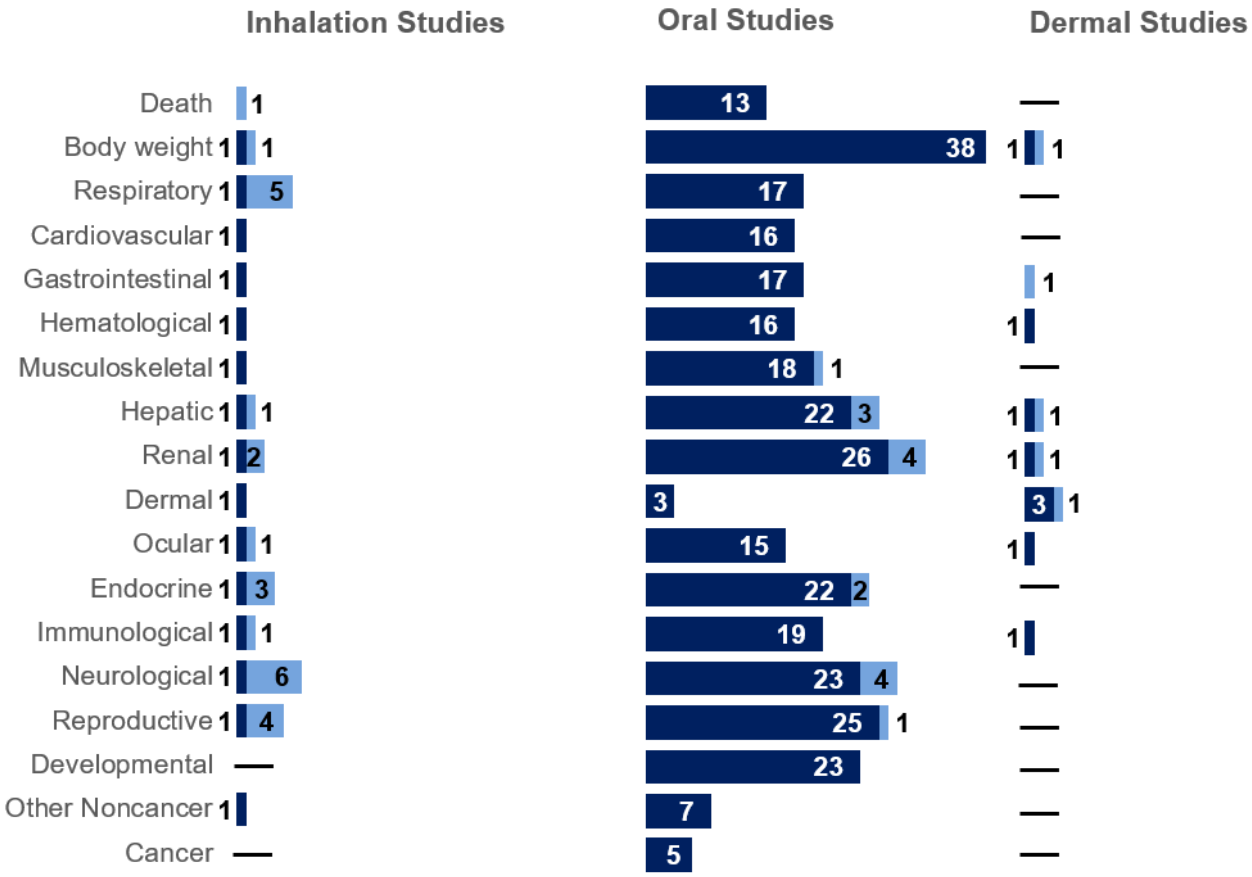
6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-D that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of 2,4-D. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

Information regarding health effects in humans following exposure to 2,4-D comes from case reports of accidental or intentional ingestion of herbicide formulations containing 2,4-D, accidental skin contact with those products by farmers and professional residential applicators, and occupational exposure during manufacture, formulation, or packaging. Information is also available from exposure of the general population. Exposure to 2,4-D during use of products containing this chemical occurred predominantly by dermal contact, but inhalation may have also occurred if a product was sprayed. The general population can be exposed by dermal contact with surfaces treated with products containing 2,4-D, by consumption of contaminated water or food, and also in house dust. No reliable estimates of quantitative exposure could be obtained from case reports, but studies have estimated exposure from measurements of 2,4-D excreted in the urine. There is no evidence suggesting that the toxicity of 2,4-D is route-specific.

Figure 6-1. Summary of Existing Health Effects Studies on 2,4-D By Route and Endpoint*

Potential body weight and kidney effects were the most studied endpoints
The majority of the studies examined oral exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2. The number of studies include those finding no effect; many studies examined more than 1 endpoint.

The database in animals is extensive. As seen in Figure 6-1, most studies in animals have been conducted by the oral route of exposure. There is more information regarding the health effects of 2,4-D following intermediate-duration exposure than regarding acute- or chronic-duration exposure.

People living near hazardous waste sites may be exposed to 2,4-D primarily via dermal contact with soil contaminated with 2,4-D, through ingestion of contaminated water, or through contaminated house dust.

6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. No information was located regarding health effects in humans following inhalation exposure to 2,4-D. No acute-duration inhalation studies in animals were located. Published inhalation studies are needed for all exposure durations. There is information regarding health effects in humans following acute-duration oral exposure to 2,4-D from case reports of intentional or accidental ingestion of herbicide formulations containing 2,4-D. Effects that have been reported following oral exposure to high amounts of 2,4-D include including tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and death (Dudley and Thapar 1972; Durakovic et al. 1992; Keller et al. 1994; Nielsen et al. 1965; Smith and Lewis 1987). Because these subjects were exposed to formulations containing 2,4-D along with other ingredients that may have contributed to the effects reported, these studies are inadequate for MRL derivation. Studies in animals provided information on lethality (Drill and Hiratzka 1953; Elo et al. 1988; Gorzinski et al. 1987; Hill and Carlisle 1947) and a wide range of endpoints including systemic effects (Dickow et al. 2000; Mattsson et al. 1997; Steiss et al. 1987), neurological effects (Mattsson et al. 1997; Steiss et al. 1987; Stürtz et al. 2008), reproductive effects (Dinamarca et al. 2007), and developmental effects (Charles et al. 2001; Chernoff et al. 1990; Collins and Williams 1971; Fofana et al. 2002; Kavlock et al. 1987; Schwetz et al. 1971). Long-term oral studies in animals suggest that the kidney is a target for 2,4-D toxicity; however, virtually no data on kidney effects were available in acute-duration studies. Therefore, an acute-duration study that examines the nature of the dose-response for kidney effects in rats or mice would be useful. Two case reports of humans acutely exposed to products containing 2,4-D by skin contact reported long-lasting

neurological alterations (Berkley and Magee 1963; Goldstein et al. 1959). A study in animals with controlled exposure to sublethal doses of 2,4-D would be useful to confirm or refute the reports in humans.

Intermediate-Duration MRLs. No studies of humans exposed to 2,4-D specifically for intermediate-duration periods (15–354 days) were located. However, it is likely that some subjects from studies mentioned below under Chronic-Duration MRLs were exposed for intermediate durations. An extensive database in animals exposed by the oral route provided information regarding systemic effects (Bortolozzi et al. 1999; Charles et al. 1996a, 1996b, 1996c; EPA 1984, 1985, 1986, 1987b, 1996; Gorzinski et al. 1987; Marty et al. 2013; Mattsson et al. 1997; Mazhar et al. 2014; Ozaki et al. 2001; Saghir et al. 2013a, 2013b; Stürtz et al. 2010; Troudi et al. 2012a, 2012b), neurological effects (Mattsson et al. 1997; Squibb et al. 1983), reproductive effects (Joshi et al. 2012), and developmental effects (Bortolozzi et al. 1999; EPA 1986; Hansen et al. 1971; Marty et al. 2013; Mazhar et al. 2014; Saghir et al. 2013a, 2013b; Stürtz et al. 2010; Troudi et al. 2012a, 2012b). These studies suggested that the kidney is a target for 2,4-D toxicity. Marty et al. (2013) reported the lowest LOAEL for kidney effects (45.3 mg/kg/day for proximal tubule degeneration); the result served as the basis for deriving an intermediate-duration oral MRL for 2,4-D. A single intermediate-duration inhalation study in animals was available for review (EPA 2008). This study examined a comprehensive number of endpoints in rats exposed to 2,4-D dusts for 28 days and established a LOAEL of 50 mg/m^3 2,4-D dusts for respiratory effects in rats; a NOAEL was not established. It would be valuable to conduct a study with lower exposure concentrations to establish a NOAEL for respiratory effects. The single study available was considered an insufficient database for MRL derivation. A report summarizing a 21-day dermal study in rabbits provided information mainly on systemic effects (EPA 1991a). A 13-week dermal study in rats or mice would be useful to examine the dose-response relationship for renal effects.

Chronic-Duration MRLs. There are numerous studies that provided information regarding exposure to 2,4-D and multiple health outcomes in humans (Beard et al. 2013; Beseler et al. 2006; Bloemen et al. 1993; Bond et al. 1988; Burns et al. 2001, 2011; Cantor et al. 1992; De Roos et al. 2003; Dhillon et al. 2008; Faustini et al. 1996; Eriksson et al. 2008; Flower et al. 2004; Fontana et al. 1998; Garry et al. 1996; Hardell and Eriksson 1999; Hardell et al. 1994; Hartge et al. 2005; Hoar et al. 1986; Hoppin et al. 2006a, 2006b, 2008; Kamel et al. 2007; Kluciński et al. 2001; Kogevinas et al. 1995; Lee et al. 2004; Lerda and Rizzi 1991; McDuffie et al. 2001; Miligi et al. 2006; Mills et al. 2005; Slager et al. 2009; Swan et al. 2003; Tanner et al. 2009; Weisenburger 1990; Weselak et al. 2007, 2008; Yang et al. 2014; Zahm et al. 1990). In these studies, exposure occurred predominantly by the dermal and inhalation routes of

exposure. Based on results from these and additional studies, there is no convincing evidence associating exposure to 2,4-D and adverse health effects in humans. As is not uncommon with epidemiological studies, limitations encountered in these studies include unreliable exposure assessment and simultaneous exposures to other chemicals. It seems prudent, however, to continue to monitor populations exposed to 2,4-D, such as pesticide applicators and manufacturers.

A limited number of chronic-duration studies in animals was available for review. These studies provided information on a wide range of endpoints in rats, mice, and dogs exposed orally to 2,4-D and suggested that the kidney is a target for 2,4-D toxicity in mice (Charles et al. 1996a; EPA 1987a, 1996; Hansen et al. 1971). Evidence of 2,4-D treatment-related degenerative/regenerative renal effects from the 2-year study in mice by Charles et al. (1996a) served as the basis for derivation of a chronic-duration oral MRL for 2,4-D. The chronic-duration oral studies also showed no evidence of carcinogenicity for 2,4-D in rats, mice, or dogs. Additional chronic-duration studies with 2,4-D do not seem necessary at this time.

Health Effects.

Genotoxicity. There are data regarding genetic effects in workers exposed to 2,4-D (i.e., Andreotti et al. 2015; Figgs et al. 2000; Garry et al. 2001; Holland et al. 2002; Hou et al. 2013), animals exposed *in vivo* (Amer and Aly 2001; Charles et al. 1999a, 1999b; Epstein et al. 1972; Kaya et al. 1999; Linnainmaa 1984; Madrigal-Bujaidar et al. 2001; Magnuson et al. 1977; Mustonen et al. 1989; Rasmuson and Svahlin 1978; Schop et al. 1990; Tripathy et al. 1993; Venkov et al. 2000; Vogel and Chandler 1974; Yilmaz and Yuksel 2005; Zettenberg et al. 1977), and *in vitro* exposure of prokaryotic cells (Charles et al. 1999b; Garret et al. 1986; Kubo et al. 2002; Mersch-Sundermann et al. 1994; Styles 1973; Venkat et al. 1995; Venkov et al. 2000; Zetterberg 1978; Zetterberg et al. 1977) and eukaryotic cells (Clausen et al. 1990; Galloway et al. 1987; González et al. 2005; Korte and Jalal 1982; Linnainmaa 1984; Maire et al. 2007; Mikalsen et al. 1990; Mustonen et al. 1986; Soloneski et al. 2007; Turkula and Jalal 1985; Venkov et al. 2000). These studies provided positive and negative results, possibly because of differences in the experimental protocols used by the different studies. Furthermore, unless a population with exposure only to 2,4-D is identified, as in a small group of workers reported by Holland et al. (2002), most studies of farmers or pesticide applicators will provide inconclusive results. However, efforts to design studies to deal with possible confounding should be encouraged.

While there have been studies on the pharmacokinetic profiles for humans (Sauerhoff et al. 1977) and animals (Van Ravenzwaay et al. 2003), it does not appear that much research has been

directed towards the 2,4-D conjugate in urine and the potential for reactive oxygen species or other metabolites that may affect hepatic or renal DNA. Although studies of this nature are important in establishing a link between metabolism, DNA damage, and potential cancer(s), available data do not suggest a genotoxic role for 2,4-D.

Reproductive. Three studies of subjects from agricultural communities did not provide convincing evidence suggesting that exposure to 2,4-D is associated with adverse reproductive effects (Arbuckle et al. 2001; Lerda and Rizzi 1991; Swan et al. 2003). Oral studies in animals provided information on gross and microscopic appearance of reproductive organs from males and females (Charles et al. 1996a, 1996b, 1996c; EPA 1984, 1985, 1986, 1987a; Gorzinski et al. 1987; Hansen et al. 1971) and fertility/reproductive indices (Dinamarca et al. 2007; Hansen et al. 1971; Joshi et al. 2012; Marty et al. 2013; Saghir et al. 2013a). These studies suggest that 2,4-D is not a reproductive toxicant. Additional reproductive toxicity studies in animals do not seem necessary at this time.

Results from *in vitro* and *in vivo* studies did not suggest that 2,4-D is an endocrine disruptor chemical (EPA 2015c, 2015d), although some studies describe behavioral effects (Bortolozzi et al. 1998, 1999, 2003; Evangelista de Duffard et al. 1995).

Developmental. A few studies are available that examined the potential association between 2,4-D and birth defects and respiratory ailments in children from subjects exposed to 2,4-D through farming activities (Garry et al. 1996; Sathyanarayana et al. 2010; Weselak et al. 2007, 2008; Yang et al. 2014). The results did not suggest a role for 2,4-D in the health outcomes examined. Studies in animals provide data on standard developmental endpoints in rodents (Charles et al. 2001; Chernoff et al. 1990; Collins and Williams 1971; EPA 1986; Fofana et al. 2000, 2002; Kavlock et al. 1987; Schwetz et al. 1971; Stürtz et al. 2010), histology of liver and bone from rat pups (Troudi et al. 2012a, 2012b), and neurobehavioral effects in rat pups (Bortolozzi et al. 1999). Some of the studies reported reduced fetal or offspring weight, in many cases accompanied by reduced maternal weight gain during pregnancy or some other maternal effect, and minor soft-tissue and skeletal anomalies, in some studies (Chernoff et al. 1990; Fofana et al. 2000, 2002; Schwetz et al. 1971). 2,4-D did not induce teratogenicity. Animal studies have demonstrated that 2,4-D can enter maternal milk and be transferred to nursing offspring, No adverse health outcomes have been reported in children whose mothers were exposed to 2,4-D

through farming activities; however, no information is available regarding levels of 2,4-D in breast milk or in neonates born to these women.

Immunotoxicity. Two studies of workers exposed to herbicides (2,4-D among them) found no evidence that 2,4-D played a role in minor immunological alterations reported in some workers (Faustini et al. 1996; Kluciński et al. 2001). An epidemiological study did find that male offspring were more prone to allergies (Weselak et al. 2007); however, the pathway for this result has not been studied. De Roos et al. (2005) found no association between rheumatoid arthritis and exposure to 2,4-D among female spouses of participants in the AHS. For the most part, studies in animals have only provided information regarding weight and gross and microscopic appearance of lymphoreticular organs and tissues from rats, mice, and dogs; no significant effects have been reported (Charles et al. 1996b, 1996c; EPA 1984, 1985, 1987a; Gorzinski et al. 1987; Hansen et al. 1971; Marty et al. 2013; Steiss et al. 1987). Only one study monitored parameters of immunocompetence in rats and reported negative results (Marty et al. 2013). 2,4-D was a respiratory allergen in mice sensitized with 2,4-D dermally and challenged with 2,4-D intratracheally (Fukuyama et al. 2009). Conduction of a Tier I screen immunology battery in B6C3F1 mice exposed to 2,4-D would be reassuring.

Neurotoxicity. There is limited information regarding neurological effects from cases of oral or dermal intoxication with commercial products containing 2,4-D (Berkley and Magee 1963; Berwick 1970; Durakovic et al. 1992; Dudley and Thapar 1972; Goldstein et al. 1959). Several studies also examined the potential association between exposure to 2,4-D and Parkinson's disease (Dhillon et al. 2008; Hancock et al. 2008; Kamel et al. 2007; Tanner et al. 2009). Only Tanner et al. (2009) reported an association between 2,4-D and Parkinson's disease. Two studies did not find an association between 2,4-D and depression among female spouses from pesticide applicators in the AHS (Beard et al. 2013; Beseler et al. 2006). Oral studies in animals did not find gross or microscopic alterations in tissues of the nervous system following exposure to 2,4-D (Charles et al. 1996b, 1996c; EPA 1984, 1987a; Gorzinski et al. 1987; Hansen et al. 1971; Marty et al. 2013; Mattsson et al. 1997; Squibb et al. 1983; Steiss et al. 1987). The available chronic-duration oral animal studies did not conduct neurobehavioral tests. However, based on available information, 2,4-D does not appear to present a particular neurotoxicity concern to humans at environmentally-relevant exposure levels.

Epidemiological and Human Dosimetry Studies. Many epidemiological studies provided information regarding exposure to 2,4-D and a wide range of health outcomes (see Chronic-Duration MRL above for references). Although some studies found that exposure to 2,4-D was positively associated with adverse outcomes, others did not. As previously noted, being significantly associated does not imply causality, although it suggests that exposure to the chemical plays some role in the health outcome assessed and that biological plausibility exists. Conduction of studies in areas where exposures to 2,4-D and other chemicals in the workplace can be adequately characterized would provide valuable information.

Biomarkers of Exposure and Effect.

Exposure. Further refinements to the methodology for estimating exposure levels from urinary levels of 2,4-D, including awareness of factors that can determine the extent of exposure, such as type of application method, glove use, repairing equipment, size of the area treated, and personal hygiene practices, would be valuable. Examining how urine collection timing in relation to exposure can affect the estimates of exposure levels also would be valuable.

Effect. There are no 2,4-D-specific effects following exposure to this substance. Effects that have been associated with acute exposure to high amounts of 2,4-D can also be induced by exposure to other chemicals or can even be caused by conditions unrelated to chemical exposures. Any research aimed at identifying a specific biomarker of effect for 2,4-D would be valuable.

Absorption, Distribution, Metabolism, and Excretion. Information is available regarding absorption, distribution, metabolism, and excretion of 2,4-D in humans and animals following oral and dermal exposure to 2,4-D (Feldmann and Maibach 1974; Griffin et al. 1997a; Harris and Solomon 1992; Khanna and Fang 1966; Kohli et al. 1974; Moody et al. 1990, 1994; Sauerhoff et al. 1977; van Ravenzwaay et al. 2003; Wester et al. 1996). These and additional studies have shown that 2,4-D is almost completely absorbed from the gastrointestinal tract, but dermal absorption is relatively low. 2,4-D distributes widely in tissues following oral exposure, does not accumulate in tissues, is subject to limited metabolism, and is eliminated via the kidneys by a mechanism that involves a saturable carrier protein. The available studies have provided a fairly good characterization of the toxicokinetics of 2,4-D and further studies do not seem necessary at this time.

PBPK models for 2,4-D in rabbits, rats, and humans have been reported (Durkin et al. 2004; Kim et al. 1994, 1995, 1996, 2001). The Kim et al. (1994, 1995, 1996, 2001) and Durkin et al. (2004) models have very different structures, although they appear to yield similar predictions of plasma elimination kinetics when optimized to the same intravenous dosing studies in rats. A particular feature of the Durkin et al. (2004) model is reversible suppression of glomerular filtration and renal blood flow at high 2,4-D concentrations, which results in dose-dependent suppression of urinary excretion. Experimental verification of reversibility of suppression of renal blood flow by 2,4-D would be useful for further validation of this model and its application to human exposures that result in high 2,4-D concentrations.

Comparative Toxicokinetics. Studies in animals have shown the existence of sex and species differences in the toxicokinetics of 2,4-D (Griffin et al. 1997a; Timchalk 2004; van Ravenzwaay et al. 2003). Differences are due principally to the species-dependent activity of the OAT1 carrier protein responsible for the secretion of 2,4-D into the urine. Species with lower capacity to excrete 2,4-D exhibit higher plasma half-life and increased susceptibility to 2,4-D toxicity, as is the case for dogs. Studies of possible genetic determinants of the OAT1 activity carrier in humans could help identify human populations with potentially increased sensitivity to 2,4-D. Studies of OAT1 activity by age, sex, health, and other conditions would be of value to help characterize acceptable exposures for susceptible populations.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

The limited information available regarding effects of 2,4-D in children comes from epidemiological studies of farming communities where 2,4-D has been used and have included monitoring of children. These studies have not provided conclusive evidence of associations between 2,4-D and adverse health outcomes in children (Flower et al. 2004; Garry et al. 1996; Metayer et al. 2013; Weselak et al. 2007, 2008; Yang et al. 2014). Continuous monitoring of children exposed to 2,4-D in farming communities is indicated to generate more data.

Animal studies have shown that 2,4-D can be transferred to the offspring through the placenta and via the mother's milk and that it distributes widely in fetal or neonatal tissues (Elo and Ylitalo 1979; Lindquist and Ullberg 1971; Marty et al. 2013; Saghir et al. 2013a, 2013b; Sandberg et al. 1996; Stürtz et al. 2000, 2006). No adverse health outcomes have been reported in children whose mothers were exposed to 2,4-D

through farming activities. Although no information is available regarding levels of 2,4-D in breast milk or in neonates born to these women, their 2,4-D exposure levels were likely many times lower than those employed in animal studies. Therefore, 2,4-D does not appear to present a particular toxicity concern to breastfeeding mothers. Monitoring of women with the highest exposures in farming communities would not likely provide valuable information.

As summarized in Section 2.17, Developmental Effects, studies in rodents have shown that, for the most part, adverse developmental effects (i.e., mainly reduced body weight in the offspring) occur at maternal dose levels that induced maternal toxicity, mainly reduced maternal weight during pregnancy. Reduced offspring weight was reported in a study in rats administered a relatively low postpartum dose of 2.5 mg 2,4-D/kg/day (Stürtz et al. 2010). Because no such effects have been reported in other studies that exposed dams to considerably higher doses, it would be useful to try to replicate those findings.

Physical and Chemical Properties. The physical-chemical properties of 2,4-D are provided in Chapter 4. Important properties such as melting point, boiling point, vapor pressure, solubility, log K_{ow} and Henry's Law constant are available. No data needs are identified.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2016, became available in 2018. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. The environmental fate and transport of 2,4-D is understood and no data needs are identified. The mobility of 2,4-D in soils is expected to be high based on measured K_{oc} values; however, detection of 2,4-D in groundwater is infrequent since it degrades rapidly in soil. Volatilization is generally considered low. Hydrolysis in acidic soils and photolysis may result in some degradation of 2,4-D. Biodegradation primarily accounts for the removal of 2,4-D from the environment.

Bioavailability from Environmental Media. 2,4-D has been detected in aquatic and terrestrial organisms (Schultz and Whitney 1974) and is therefore bioavailable to some extent from environmental media; however, elimination from the organisms was rapid. Aerobic biodegradation reduces its bioavailability. No data needs are identified.

Food Chain Bioaccumulation. Measured BCFs of 2,4-D in fish suggest that bioaccumulation in aquatic organisms is not high. No data needs are identified.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of 2,4-D in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 2,4-D in the environment can be used in combination with the known body burden of 2,4-D to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Humans are exposed to 2,4-D mainly by dermal exposure during application as an herbicide. Populations may also be exposed by transport of 2,4-D into residential homes from agricultural spray drift, volatilization, soil or dust resuspension, track-in on shoes, and on clothing. Adequate biomonitoring data are available to assess 2,4-D exposure to the general population of the United States. Continued monitoring of the general U.S. population through the NHANES program can provide information on the trend of exposure to 2,4-D and identify subsets in the population with the highest levels of exposure.

Exposures of Children. Children are exposed to 2,4-D mainly by dermal exposure to residue transported into homes from applicators and from direct contact with treated residential lawns. Adequate biomonitoring data are available to assess 2,4-D exposure to children of the United States. Continued monitoring through the NHANES program is needed in order to understand future exposures. Additional research on exposures of neonates and young children of workers who handle 2,4-D is needed and justifiable. No human data were located regarding 2,4-D in breast milk and this is a data need.

6.3 ONGOING STUDIES

The following ongoing research pertaining to 2,4-D (Table 6-1) was identified in the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2018):

Table 6-1. Ongoing Studies on 2,4-D

Investigator	Affiliation	Research description	Sponsor
Laura Beane Freeman	Division of Cancer Epidemiology and Genetics of NCI	Investigation of potential associations between exposure to pesticides (2,4-D among them) and a wide range of health endpoints in participants in the AHS	NCI

Table 6-1. Ongoing Studies on 2,4-D

Investigator	Affiliation	Research description	Sponsor
Jian Feng	VA Western New York Healthcare System	The interaction of parkin and environmental toxins (2,4-D among them) in Parkinson's disease	VA
Melissa Friesen	Division of Cancer Epidemiology and Genetics of NCI	Assessment of occupational exposure to a variety of substances including 2,4-D	NCI
Jane Hoppin	Biology, Schools of Arts and Sciences, North Carolina State University Raleigh	Environmental pesticide exposure (includes 2,4-D) and respiratory outcomes in women and children	NIEHS
Timothy D. Howard	Obstetrics & Gynecology, Schools of Medicine, Wake Forest University Health Sciences	Human pesticide exposure (includes 2,4-D) and epigenetic changes in sperm DNA	NIEHS
Lee S. Newman	Public Health & Preventive Medicine, Schools of Public Health, University of Colorado Denver	Etiologic and mechanistic factors underlying chronic kidney disease in Guatemalan sugarcane workers	NIEHS
Steven D. Stellman	Eunice Kennedy Shriver NICHD, Foundation for Worker/Veteran Environmental Health	Agent orange (2,4-D is a component) and adverse birth outcomes: a re-examination	NICHD

2,4-D = 2,4-dichlorophenoxyacetic acid; AHS = Agricultural Health Study; DNA = deoxyribonucleic acid; NCI = National Institutes of Health; NICHD = National Institute of Child Health and Human Development; NIEHS = National Institute of Environmental Health Sciences; VA = Veteran's Affairs

Source: RePORTER 2018

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding 2,4-D in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for 2,4-D.

Table 7-1. Regulations and Guidelines Applicable to 2,4-D

Agency	Description	Information	Reference
	Air		
EPA	RfC	No data	IRIS 2002
WHO	Air quality guidelines	Not listed	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2018
	1-Day health advisory (10-kg child)	1 mg/L	
	10-Day health advisory (10-kg child)	0.3 mg/L	
	DWEL	0.2 mg/L	
	Lifetime health advisory	No data	
	10^{-4} Cancer risk	No data	
	National primary drinking water regulations		EPA 2009
	MCL	0.07 mg/L	
	PHG	0.07 mg/L	
	OPP's RfD	0.21 mg/kg/day	EPA 2017
WHO	Drinking water quality guidelines		WHO 2017
	Guideline value	0.03 mg/L[a]	
	ADI	0–0.01 mg/kg body weight[b]	
FDA	Substances added to food	Not listed[c]	FDA 2018
	Allowable level in bottled water	0.07 mg/L	FDA 2017
	Cancer		
HHS	Carcinogenicity classification	No data	NTP 2016
EPA	Carcinogenicity classification	Group D[d]	EPA 2005a
IARC	Carcinogenicity classification	Group 2B[e]	IARC 2018

Table 7-1. Regulations and Guidelines Applicable to 2,4-D

Agency	Description	Information	Reference
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, construction, and shipyards	10 mg/m^3	OSHA 2018a, OSHA 2018b, OSHA 2018c
NIOSH	REL (up to 10-hour TWA)	10 mg/m^3	NIOSH 2016
	IDLH	100 mg/m^3	NIOSH 1994
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2016b
DOE	PACs-air		DOE 2018b
	PAC-1[f]	30 mg/m^3	
	PAC-2[f]	94 mg/m^3	
	PAC-3[f]	500 mg/m^3	

[a]Applies to free acid.
[b]For the sum of 2,4-D and its salts and esters.
[c]The Substances Added to Food inventory (formerly EAFUS) contains food additives and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in foods prior to September 6, 1958, substances that are listed in FDA regulations as prohibited from use in food or as delisted color additives, and some substances "no longer FEMA GRAS."
[d]Group D: not classifiable as to human carcinogenicity.
[e]Group 2B: possibly carcinogenic to humans
[f]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2018a).

ADI = acceptable daily intake; AEGL = acute exposure guideline levels; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers Association; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; JECFA = Joint Expert Committee on Food Additives; LOAEL = lowest-observed-adverse-effect level; MCL = maximum contaminant level; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OPP = Office of Pesticide Programs; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PEL = permissible exposure limit; PHG = public health goal; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; T4 = thyroxine; TWA = time-weighted average; WHO = World Health Organization

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-D
CAS Numbers: 94-75-7
Date: July 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: An acute-duration inhalation MRL was not derived for 2,4-D.

Rationale for Not Deriving an MRL: No acute-duration inhalation data were available for review.

Agency Contacts (Chemical Managers): Obaid Faroon

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-D
CAS Numbers: 94-75-7
Date: July 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: An intermediate-duration inhalation MRL was not derived for 2,4-D.

Rationale for Not Deriving an MRL: Only one inhalation study in animals was available for review. In that study, male and female rats were exposed nose-only 6 hours/day, 5 days/week for 28 days to 2,4-D dusts in target concentrations of 0, 50, 100, 300, and 1,000 mg/m^3 (EPA 2008). After termination of exposure, controls and rats from the highest exposure concentration group were kept for a 4-week recovery period to assess reversibility of the effects. A significant reduction in reticulocytes occurred in males and females at 2,4-D exposure concentrations ≥300 mg/m^3 and a significant increase in serum alkaline phosphatase was reported in females at ≥300 mg/m^3. Histopathologic examination of a comprehensive set of organs and tissues revealed no sign of 2,4-D exposure-related systemic effects. The most salient effect was the occurrence of squamous/squamoid epithelial metaplasia with hyperkeratosis in the larynx of all exposed groups, with increasing severity as the exposure concentration increased. The lesions persisted during the recovery period, but with reduced severity. Therefore, the exposure concentration of 50 mg/m^3 represents the study LOAEL, a portal-of-entry LOAEL. Although this is a well-conducted study that examined a comprehensive number of end points, the database is insufficient for MRL derivation. It would be important to determine a NOAEL for the portal-of-entry effects.

Agency Contacts (Chemical Managers): Obaid Faroon

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-D
CAS Numbers: 94-75-7
Date: July 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: A chronic-duration inhalation MRL was not derived for 2,4-D

Rationale for Not Deriving an MRL: No chronic-duration inhalation data were available for review.

Agency Contacts (Chemical Managers): Obaid Faroon

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-D
CAS Numbers: 94-75-7
Date: July 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: An acute-duration oral MRL was not derived for 2,4-D.

Rationale for Not Deriving an MRL: No adequate acute-duration human data were located. Information regarding health effects in humans following acute-duration exposure to 2,4-D is limited to case reports of intentional or accidental ingestion of herbicide formulations containing 2,4-D. Effects that have been reported following oral exposure to high amounts of 2,4-D include tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and death (Dudley et al. 1972; Durakovic et al. 1992; Keller et al. 1994; Nielsen et al. 1965; Smith and Lewis 1987). While some of these studies provided estimates of amounts of 2,4-D ingested, the reported effects represent the result of exposure to a chemical mixture consisting of 2,4-D and other substances present in the commercial formulations (i.e., solvents, other herbicides), which is the exposure that most humans experience. Yet, the common exposure reported across studies was to 2,4-D. In two studies designed to evaluate the pharmacokinetics of 2,4-D in volunteers, administration of 5 mg 2,4-D/kg once in a gelatin capsule resulted in no ill effects during 144–168 hours post-dosing (Kohli et al. 1974; Sauerhoff et al. 1977). The information available in humans is inadequate for MRL derivation.

Two animal studies defined LOAELs of 50 mg/kg/day. In one of these studies, doses of 50 mg/kg/day (lowest dose tested) induced significant weight loss in pregnant Wistar rats when administered by gavage in water on GDs 6–15 (Fofana et al. 2000). It is not totally clear, however, whether the investigators meant that the final weight was lower than the starting weight or whether treated rats just gained less weight than control rats. In another developmental study, administration of 2,4-D at 50 mg/kg/day by gavage in corn oil to pregnant Sprague-Dawley rats, also on GDs 6–15, did not affect maternal weight (terminal weight similar in treated and controls), but induced a statistically significant reduction in fetal weight (approximately 7%) measured on GD 20 and increased the incidence of some soft-tissue anomalies and skeletal malformations; the NOAEL was 25 mg/kg/day (Schwetz et al. 1971). Long-term oral studies suggest that the kidney is a target for 2,4-D toxicity; however, only one acute-duration study conducted microscopic examinations of the kidneys. The paucity of information regarding the effects of acute-duration oral exposure to 2,4-D in experimental animals precludes deriving an acute-duration oral MRL for 2,4-D.

Agency Contacts (Chemical Managers): Obaid Faroon

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-D
CAS Numbers: 94-75-7
Date: July 2020
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: 0.2 mg/kg/day
Critical Effect: Increased kidney weight, histopathologic kidney lesions
Reference: Marty et al. 2013
Point of Departure: 16.6 mg/kg/day (NOAEL)
Uncertainty Factor: 100
LSE Graph Key: 33
Species: Rat

MRL Summary: An MRL of 0.2 mg/kg/day has been derived for intermediate-duration oral exposure to 2,4-D based on a NOAEL of 16.6 mg/kg/day and a LOAEL of 45.3 mg/kg/day for increased kidney weight and slight proximal tubule degeneration in the kidney of male Sprague-Dawley rats receiving 2,4-D from food for up to 11 weeks (Marty et al. 2013).

Selection of the Critical Effect: No human data were located. Available animal data identify the kidney and developmental endpoints as most sensitive to 2,4-D toxicity. Available results from intermediate-duration oral treatment of dogs were not considered an appropriate basis for MRL derivation because dogs appear to be more sensitive than rodents or humans due to a significantly lower capacity to eliminate 2,4-D via the kidneys (Timchalk 2004). Table A-1 summarizes study results for renal effects and for developmental effects considered potential points of departure for deriving an intermediate-duration oral MRL for 2,4-D.

Table A-1. Summary of Potential Candidate Critical Effects for Deriving an Intermediate-Duration Oral MRL for 2,4-D

Species	Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Renal effects					
F344 rat	13 weeks (F)	1	5	Increased homogeneity and altered tinctorial properties in cytoplasm; fine vacuolization of cytoplasm in renal cortex	EPA 1984
B6C3F1 mouse	13 weeks (F)	5	15	M, F: Increased homogeneity, altered tinctorial properties in cytoplasm or renal cortex; M: decreased vacuolization in renal cortex	EPA 1984
B6C3F1 mouse	52 weeks (F)	1 M 45 F	15 M	Increased cytoplasmic homogeneity of renal tubular epithelium due to reduction of cytoplasmic vacuoles	EPA 1987a
F344 rat	40 weeks (F)	5 M	20 M	Increased incidence and severity of fine vacuolization in cytoplasm of renal cortex	EPA 1987b

Table A-1. Summary of Potential Candidate Critical Effects for Deriving an Intermediate-Duration Oral MRL for 2,4-D

Species	Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
F344 rat	52 weeks (F)	15 F	45 F	F: Increased incidence and severity of fine vacuolization in cytoplasm of renal cortex	EPA 1985
Crl:CD(SD) rat	M: 11–13 weeks F: 10–12 weeks (F)	16.6 M 45.2[a] F	45.3 M	Increased relative kidney weight, increased incidence of multifocal slight degeneration of proximal convoluted tubules in outer stripe of outer zone of medulla	Marty et al. 2013
Crl:CD(SD) rat	PPDs 21–70[b] (F)	28.4 M 28.8 F	76.6 M 57.9 F	F1 parental males and females: kidney lesions similar to those of P1 males	Marty et al. 2013
F344 rat	13 weeks (F)	15	60	M: Increased epithelial cytoplasmic homogeneity, multifocal slight degeneration in descending proximal tubules F: Increased cytoplasmic vacuolization in proximal convoluted tubules	Gorzinski et al. 1987
B6C3F1 mouse	12 months (F)	5 M	62.5 M	5% increased kidney weight; degeneration/regeneration in descending limb of proximal tubules; vacuolation of proximal tubules	Charles et al. 1996a; EPA 1996b
F344 rat	52 weeks (F)	5	75	Degeneration of proximal convoluted tubules	Charles et al. 1996a; EPA 1996a
Crl:CD(SD) rat	M: 71 days F: 96 days (F)	50 M	75 M	Slight multifocal degeneration of proximal convoluted tubules in outer stripe of outer zone of medulla of males	Saghir et al. 2013a
F344 rat	13 weeks (F)	15	100	21 and 12% increased relative kidney weight in males and females, respectively	Charles et al. 1996b
Developmental effects					
Crl:CD(SD) rat	42 days (GD 1–LD 21 (F)	24.7 F	49.4 F	9% depressed PPD 22 F1a male pup body weight (based on estimated TWA parental female dose for GD 0–LD 14)	Marty et al. 2013
Crl:CD(SD) rat	M: 80 days F: 95 days (F)	25	50	13–23% depressed pup weight during PPDs 14–21	Saghir et al. 2013a, 2013b

Table A-1. Summary of Potential Candidate Critical Effects for Deriving an Intermediate-Duration Oral MRL for 2,4-D

Species	Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
F344 rat	40 weeks (F)	32	110	24% depressed PPD 21 pup body weight	EPA 1986

[a]The P1 female dose is a TWA dose calculated from reported dose estimates for three separate time periods (29 days premating, 21 days of gestation, and the first 14 days of lactation).
[b]Rats had been exposed via their mothers during gestation and lactation as well.

F = female(s); (F) = food; GD = gestation day; LD = lactation day; LOAEL = lowest observed adverse effect level; M = male(s); NOAEL = no-observed-adverse-effect level; PPD = postparturition day; TWA = time-weighted average

Selection of the Principal Study: Available Data Evaluation Records (DERs) from early studies submitted to EPA (EPA 1984, 1985, 1987a, 1987b) provide inadequate descriptions of the kidney lesions reported. Thus, the degenerative nature of the described lesions is in question. Therefore, these studies were not considered as candidate principal studies for deriving an intermediate-duration oral MRL for 2,4-D.

Three studies provide corroborative evidence of degenerative changes in renal proximal tubules within the outer stripe of the outer zone of the medulla of rats (Gorzinski et al. 1987; Marty et al. 2013; Saghir et al. 2013a). Each of these studies described similar histologic changes that included shrinking, crowding and basophilic staining of epithelial cells, and basement membrane thickening.

> Marty et al. (2013, and as more fully described in MRID4792101): "This degenerative lesion involving the proximal convoluted tubules in the outer strip of the outer zone of the medulla, was multifocal in distribution and slight in degree. This lesion was primarily characterized by tubular epithelial cells, which were basophilic staining and had nuclei that were crowded together due to a decrease in the amount of cytoplasm (eosinophilic staining). Pyknotic nuclei were also occasionally noted in these tubules. Remaining portions of these tubular profiles appeared normal. **Affected tubules also had focally thickened basement membranes**, adjacent interstitial fibrous connective tissue proliferation and a mononuclear inflammatory cell infiltrate".

> Saghir et al. (2013a, study submitted to EPA as MRID47417901): "This was a degenerative multifocal lesion involving the proximal convoluted tubules in the outer strip of the outer zone of the medulla and was very slight or slight in degree of severity. This lesion was primarily characterized by tubular epithelial cells, which were basophilic staining and had nuclei that were crowded together due to a decrease in the amount of cytoplasm (eosinophilic staining). Pyknotic nuclei were also occasionally noted in these tubules. Remaining portions of these tubular profiles appeared normal. **Affected tubules also had focally thickened basement membranes**, adjacent interstitial fibrous connective tissue proliferation and a mononuclear inflammatory cell infiltrate".

> Gorzinski et al. (1987): "…multifocal degeneration in the descending part of the proximal tubules...basophilic epithelial cells that were crowded because of decreased cytoplasm...**accompanied with thickened basement membranes** and interstitial fibrosis".

None of the above studies found evidence of cell death or tubule destruction (e.g., apoptosis, necrosis), even at the highest dose, and no studies found evidence of renal functional impairment. Therefore, the

histological changes observed can be considered to be minimally severe (pre-clinical) indications of degenerative changes that might become more severe at higher doses and/or longer exposure durations.

Two-year oral studies in rats (Charles et al. 1996a; EPA 1996b) and mice (Charles et al. 1996a; EPA 1996a) included histopathological evaluations at 1-year interim sacrifice. The rat study identified a NOAEL of 5 mg/kg/day and a LOAEL of 75 mg/kg/day for degeneration in proximal tubules. The mouse study identified a NOAEL of 5 mg/kg/day and a LOAEL of 62.5 mg/kg/day for degeneration/ regeneration in descending limb of proximal tubules in the male mice. There was no mention of basement membrane thickening in the publicly-available summaries of these studies or the unpublished MRID studies. Therefore, these studies were not considered as candidate principal studies for MRL derivation.

Kidney results for male rats from the studies of Gorzinski et al. (1987), Saghir et al. (2013a), and Marty et al. (2013) are summarized in Tables A-2, A-3, and A-4, respectively. Gorzinski et al. (1987) identified a NOAEL of 15 mg/kg/day and La OAEL of 60 mg/kg/day for multifocal degeneration of the descending proximal tubule. The severity of the lesions was graded as slight at 60 mg/kg/day and moderate at ≥100 mg/kg/day. The Saghir et al. (2013a) and Marty et al. (2013) studies reported very slight multifocal degeneration of the convoluted tubules in male rats at low dose levels. The very slight generation was not considered adverse because it was not associated with alterations in kidney weight or evidence of renal impairment. Additionally, a high incidence (9/10) of very slight degeneration was observed in the control group of the Marty et al. (2013) study. The slight degeneration observed at higher doses was considered adverse. The NOAEL and LOAEL values were 50 and 75 mg/kg/day, respectively, in the Saghir et al. (2013a) study and 16.6 and 45.3 mg/kg/day, respectively, in the Marty et al. (2013) study.

Table A-2. Selected Kidney Results from Male F344 Rats Receiving 2,4-D from the Diet for 11 Weeks

Reported 2,4-D dose (mg/kg/day)	0	15	60	100	150
Degeneration descending proximal tubule					
Slight multifocal degeneration	0/10	2/10	8/10[a]	1/10	0/10
Moderate multifocal degeneration	0/10	0/10	2/10	9/10[a]	10/10[a]

[a]Statistically different from control mean by Fischer's exact test ($p<0.01$) performed by ATSDR.

Source: Gorzinski et al. 1987

Table A-3. Selected Kidney Results from Male Crl:CD(SD) Rats Receiving 2,4-D from the Diet for 11 Weeks

Dietary concentration (ppm)	0	100	400	1,000/800	2,000/1,200	1,600
Estimated dose (mg/kg/day)[a]	0	6	25	50	75	100
Kidney weight (g/100 g body weight)	0.68	0.72	0.71	0.74	0.75	0.81[b]
Degeneration proximal convoluted tubule						
Very slight multifocal degeneration	1/10	1/10	6/10[c]	8/10[c]	4/10[d]	7/10[c]
Slight multifocal degeneration	0/10	0/10	0/10	0/10	4/10[c]	1/10

[a]Estimated dose for prebreeding period only.
[b]Statistically different from control mean by Dunnett's test (α=0.05).
[c]Statistically different from control mean by Fischer's exact test (p<0.01) performed by ATSDR.
[d]Statistically different from control mean by Fischer's exact test (p<0.05) performed by ATSDR.

Source: Saghir et al. 2013a

Table A-4. Selected Kidney Results from Male Crl:CD(SD) Rats Receiving Technical Grade 2,4-D from the Diet up to 13 Weeks

Dietary concentration (ppm)	0	100	300	800
Estimated dose (mg/kg/day)	0	5.51	16.6	45.3
Degeneration proximal convoluted tubule				
Very slight multifocal degeneration	9/10	4/10	6/10	3/11
Slight multifocal degeneration	1/10	0/10	1/10	8/11[a]
Kidney weight (g/100 g body weight)	0.662	0.686	0.685	0.734[a]

[a]Statistically different from control mean by Fisher's exact test (p<0.01)
[a]Statistically different from control mean by Dunnett's test (α=0.05).

Source: Marty et al. 2013

Collectively, the studies of Gorzinski et al. (1987), Marty et al. (2013), and Saghir et al. (2013a) support a LOAEL range of 45–75 mg/kg/day for slight degenerative changes in the renal proximal tubule, and a NOAEL range of 6–16.6 mg/kg/day. Among the three studies providing adequate description of degenerative changes in the renal proximal tubule (Gorzinski et al. 1987; Marty et al. 2013; Saghir et al. 2013a), the study of Marty et al. (2013) identified the lowest LOAEL (45.3 mg/kg/day) for increased kidney weight and slight degeneration of the proximal tubule in the parental male rats administered 2,4-D in the diet for up to 13 weeks; the corresponding NOAEL for this effect was 16.6 mg/kg/day. Therefore, the Marty et al. (2013) study was selected as the principal study for deriving an intermediate-duration oral MRL for 2,4-D.

Summary of the Principal Study:

Marty MS, Neal BH, Zablotny CL, et al. 2013. An F1-extended one-generation reproductive toxicity study in Crl:CD(SD) rats with 2,4-dichlorophenoxyacetic acid. Toxicol Sci 136(2):527-547. 10.1093/toxsci/kft213.

In the principal study, male Sprague-Dawley rats (27/group) were fed a diet containing 0, 100, 300, or 800 ppm 2,4-D (97.85% pure) for 4 weeks premating, up to 2 weeks of mating, and up to 7 weeks postmating. This diet provided estimated 2,4-D doses of 0, 5.5, 16.6, and 45.3 mg/kg/day, respectively, to the parental males. Female rats (27/group) were fed a diet containing 0, 100, 300, or 600 ppm 2,4-D for 29 days premating and through gestation (21 days) and lactation (21 days). Estimated 2,4-D doses to the parental females in the 0, 100, 300, and 800 ppm dietary groups were:

- 0, 6.97, 20.6, and 40.2 mg/kg/day during premating;
- 0, 7.37, 21.9, and 43.7 mg/kg/day for GDs 0–20;
- 0, 10.7, 32.4, and 64.5 g/kg/day for LDs 1–7; and
- 0, 8.4, 25.5, and 51.5 mg/kg/day for LDs 7–14.

The P1 generation was evaluated for systemic toxicity as well as reproductive toxicity.

Body weight was reduced in high-dose parental females during lactation, but no data were shown. Exposure to 2,4-D increased absolute and relative kidney weight (11–13%) in high-dose parental males and increased relative kidney weight (11%) in one set of F1 high-dose females. Renal lesions consisting of very slight to slight degeneration of the proximal convoluted tubules in the outer zone of the medulla were seen in high-dose parental males and in two sets of adult F1 females. There were no treatment-related lesions in other tissues (tissues not specified in paper). Renal lesions appeared more severe in males than in females. Nonsignificant decreased T4 and T3 and increased TSH were seen in high-dose satellite females on GD 17. Three out of 12 females had histological alterations consisting of smaller thyroid follicles with small vacuoles in the colloid suggesting colloid resorption. There were no adverse pathological alterations or changes in LD 21 dams, suggesting that the changes were transient and were therefore considered adaptive, although the changes were considered exposure-related.

The kidney effects in the parental male rats (increased incidence of slight degeneration in proximal tubules and increased kidney weight at 45.3 mg/kg/day) in the study of Marty et al. (2013) represent the most sensitive endpoint of intermediate-duration oral exposure to 2,4-D.

Selection of the Point of Departure for the MRL: A benchmark dose (BMD) approach was initially considered to derive an intermediate-duration oral MRL for 2,4-D based on incidences of kidney lesions (slight multifocal degeneration in the proximal convoluted tubule) in the male rats of Marty et al. (2013). However, as shown in Table A-4, an increased incidence of slight degeneration in the proximal tubules of the male rats was only observed in the high-dose group (incidence of 8/11 at 45.3 mg/kg/day versus 1/10, 0/10, and 1/10 for controls, 5.51, and 16.6 mg/kg/day groups, respectively). A dataset exhibiting a response only at the highest dose level would likely provide limited information regarding the shape of a dose-response curve. Therefore, the NOAEL of 16.6 mg/kg/day was considered the most appropriate potential point of departure for the male rat kidney lesion data from Marty et al. (2013).

Relative kidney weight in the male rats of Marty et al. (2013) was amenable to BMD analysis using the mean (and standard deviation) kidney weight data reported in an unpublished version of this study (MRID4792101). All continuous variable models in the benchmark dose software (BMDS) Version 3.1 were fit to the mean relative kidney weight data using a benchmark response (BMR) of one standard deviation from control in the absence of a rationale for using an alternative BMR.

The resulting potential point of departure ($BMDL_{1SD}$ of 34.12 mg/kg/day from the best-fitting model) was higher than the point of departure using a NOAEL/LOAEL approach (NOAEL of 16.6 mg/kg/day). Therefore, the NOAEL/LOAEL approach was taken to derive an intermediate-duration oral MRL.

Calculations

Intermittent Exposure: Not applicable.

Uncertainty Factor: The NOAEL of 16.6 mg/kg/day was divided by a total uncertainty factor of 100:

- 10 for extrapolation from animals to humans
- 10 for human variability

MRL = NOAEL ÷ uncertainty factors
MRL = 16.6 mg/kg/day ÷ (10 x 10) = 0.2 mg/kg/day.

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Kidney lesions (degeneration of the descending portion of proximal convoluted tubules) were reported in male and female rats receiving 2,4-D from food at 75 mg/kg/day for 12 months (interim sacrifice in a 2-year study); the NOAEL was 5 mg/kg/day (Charles et al. 1996a; EPA 1996a). Both 12-month interim and 2-year terminal sacrifices of male B6C3F1 mice revealed degenerative kidney lesions in the descending portion of proximal convoluted at a 2,4-D dose level of 62.5 mg/kg/day (Charles et al. 1996a; EPA 1996b). The NOAEL in the mouse study was 5 mg/kg/day.

Agency Contacts (Chemical Managers): Obaid Faroon

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 2,4-D
CAS Numbers: 94-75-7
Date: July 2020
Profile Status: Final
Route: Oral
Duration: Chronic
MRL: 0.2 mg/kg/day
Critical Effect: Proximal tubule degeneration/regeneration in kidney
Reference: Charles et al. 1996a; EPA 1996b
Point of Departure: 16.66 mg/kg/day ($BMDL_{10}$)
Uncertainty Factor: 100
LSE Graph Key: 59
Species: Mouse

MRL Summary: An MRL of 0.2 mg/kg/day has been derived for chronic-duration oral exposure to 2,4-D based on a $BMDL_{10}$ of 16.66 mg/kg/day for proximal tubule degeneration/regeneration in the kidney of male B6C3F1 mice receiving 2,4-D from food for up to 2 years (Charles et al. 1996a; EPA 1996b).

Selection of the Critical Effect: Table A-5 summarizes the potential candidate critical effects for deriving a chronic-duration oral MRL for 2,4-D.

Table A-5. Summary of Potential Candidate Critical Effects for Deriving a Chronic-Duration Oral MRL for 2,4-D

Species	Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Body weight effects					
F344 rat	2 years (F)	5	75	19% depressed weight gain in females	Charles et al. 1996a; EPA 1996a
Hematological effects					
F344 rat	2 years (F)	5	75	M: decreased platelets F: decreases in platelets, RBC count, hematocrit	Charles et al. 1996a; EPA 1996a
Renal effects					
B6C3F1 mouse	2 years (F)	5 M 5 F	62.5 M 150 F	Proximal tubule degeneration/regeneration	Charles et al. 1996a; EPA 1996b
Endocrine effects					
F344 rat	2 years (F)	5	75	M, F: decreased serum T4 F: increased thyroid weight	Charles et al. 1996a; EPA 1996b

(F) = food; F = female(s); LOAEL = lowest observed adverse effect level; M = male(s); NOAEL = no-observed-adverse-effect level; RBC = red blood cell; T4 = thyroxine

Selection of the Principal Study: No adequate human data were located. Chronic-duration oral studies in rats (Charles et al. 1996a; EPA 1996a; Hansen et al. 1971), mice (Charles et al. 1996a; EPA 1987a, 1996b), and dogs (Hansen et al. 1971) were available for review. In the dog study (Hansen et al. 1971),

no adverse effects were observed at the highest 2,4-D dose level tested (10 mg/kg/day). Furthermore, as previously stated, dogs appear to be more sensitive to 2,4-D toxicity than other species (including humans) due to a significantly lower capacity to eliminate 2,4-D via the kidneys (Timchalk 2004). Therefore, the dog study was not considered as a potential principal study for deriving a chronic-duration oral MRL for 2,4-D.

A 2-year bioassay in F-344 rats defined an overall NOAEL of 5 mg 2,4-D/kg/day for organs and tissue histopathology and hematological and clinical chemistry parameters (Charles et al. 1996a; EPA 1996a). An oral dose level of 75 mg/kg/day resulted in decreased platelet and erythrocyte counts and hematocrit in female rats (data not shown), increased serum ALT in male rats, and decreased serum T4 in both sexes. Histological alterations were noted at 150 mg/kg/day and consisted of a nonsignificant increase in parafollicular cell nodular hyperplasia in the thyroid from females and minimal panlobular tinctorial properties in the liver from males and females. No clear treatment-related histological alterations were observed in the kidneys at 2,4-D doses of 5, 75, or 150 mg/kg/day. An earlier study did not report treatment-related alterations in organs and tissues from Osborne-Mendel rats receiving 2,4-D from food for 2 years at approximately 92 mg/kg/day (Hansen et al. 1971).

In a 2-year mouse study submitted to EPA, male B6C3F1 mice receiving 2,4-D from food for 2 years at 15 mg/kg/day exhibited significantly increased incidence of cytoplasmic homogeneity in the renal tubular epithelium; this was attributed to a reduction of cytoplasmic vacuoles normally present in the cytoplasm of epithelial cells. No significant increase was seen at 1 mg/kg/day. The available DER from the study (EPA 1987a) provides an inadequate description of the kidney lesions reported. Thus, the degenerative nature of the described lesions is in question. Therefore, this study was not considered as a candidate for deriving a chronic-duration oral MRL for 2,4-D.

In another 2-year mouse study (Charles et al. 1996a; EPA 1996b), a significant increase in minimal degeneration with regeneration of the descending portion of the proximal tubules was reported for male B6C3F1 mice receiving 2,4-D from food at ≥62.5 mg/kg/day; the NOAEL was 5 mg/kg/day. Reduced vacuolization of the cytoplasm in tubular cells of the male mice dosed at ≥62.5 mg/kg/day was noted as well. Because of the unclear biological significance of the reduced vacuolization of the cytoplasm in tubular cells, the degeneration/regeneration change in the proximal tubule of the male mice represents a more toxicologically relevant endpoint for MRL derivation. No other treatment-related histological alterations in organs or tissues or in hematology tests were reported in mice.

The 2-year mouse study (Charles et al. 1996a; EPA 1996b) was selected as the principal study for deriving a chronic-duration oral MRL for 2,4-D because it identified the lowest reliable LOAEL for kidney effects that represent the most sensitive target of 2,4-D toxicity.

Summary of the Principal Study:

Charles JM, Bond DM, Jeffries TK, et al. 1996a. Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxyacetic acid in rodents. Fundam Appl Toxicol 33(2):166-172.

EPA. 1996b. Data Evaluation Record. Carcinogenicity study – mice. 2,4-Dichlorophenoxyacetic acid (2,4-D) [MRIDs evaluated: 43879801 & 43597201]

Groups of B6C3F1 mice (60/sex/group) were fed a diet for 2 years that provided 2,4-D at 0, 5, 62.5, and 125 mg/kg/day for males and 0, 5, 150, and 300 mg/kg/day for females (Charles et al. 1996a; EPA 1996b). Ten mice per group were killed at 1 year for examination. Endpoints monitored included overt toxicity, morbidity, and lethality at least twice weekly. Body weight, clinical signs, and food consumption were determined weekly for the first 13 weeks and monthly thereafter. Ophthalmoscopic

examination was performed at the beginning and at the end of the study. Hematology testing was conducted during weeks 52, 78, and 104. All survivors were subjected to necropsy after 52 or 104 weeks of treatment. Major organ weights were recorded; all major organs and tissues were examined microscopically.

Dosing with 2,4-D did not significantly affect survival rate. There were no treatment-related changes in clinical appearance or behavior. Body weight gain was not significantly affected by dosing with 2,4-D. Results from hematological tests were unremarkable. Ophthalmology tests did not show treatment-related alterations. Significant changes in organ weights were limited to an increase in relative kidney weight in males and females at 125 mg/kg/day. Treatment-related histological alterations were restricted to the kidneys and consisted of a significant increase in the incidence of degeneration/regeneration in the descending limb of the proximal tubule of the kidneys in males and females at 62.5 and 125 mg/kg/day and increased incidence of vacuolization of the proximal tubule in males at 62.5 and 125 mg/kg/day.

Selection of the Point of Departure for the MRL: The incidence data for degeneration with regeneration of the descending portion of the proximal tubules in the male mice (Table A-6) were analyzed using all available dichotomous models in the EPA BMDS (version 3.1), the extra risk option, and a BMR of 10% change from controls.

Table A-6. Incidence of Degeneration/Regeneration in the Descending Limb of the Proximal Tubules of Male B6C3F1 Mice Administered 2,4-D in the Food for 2 Years

Dose (mg/kg/day)	Mice/group	Incidence
0	50	0
5	50	0
62.5	50	25[a]
125	50	48[a]

[a]$p \leq 0.05$.

Sources: Charles et al. 1996a; EPA 1996b

Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL (95% lower confidence limit on the BMD) was selected as the point of departure when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) was chosen. As shown in Table A-7, all models except the Multistage (1-degree) and Dichotomous Hill models provided an adequate fit to the dataset.

Table A-7. Model Predictions for Incidence of Degeneration/Regeneration in the Descending Limb of the Proximal Tubules of Male Mice Administered 2,4-D in the Food for 2 Years

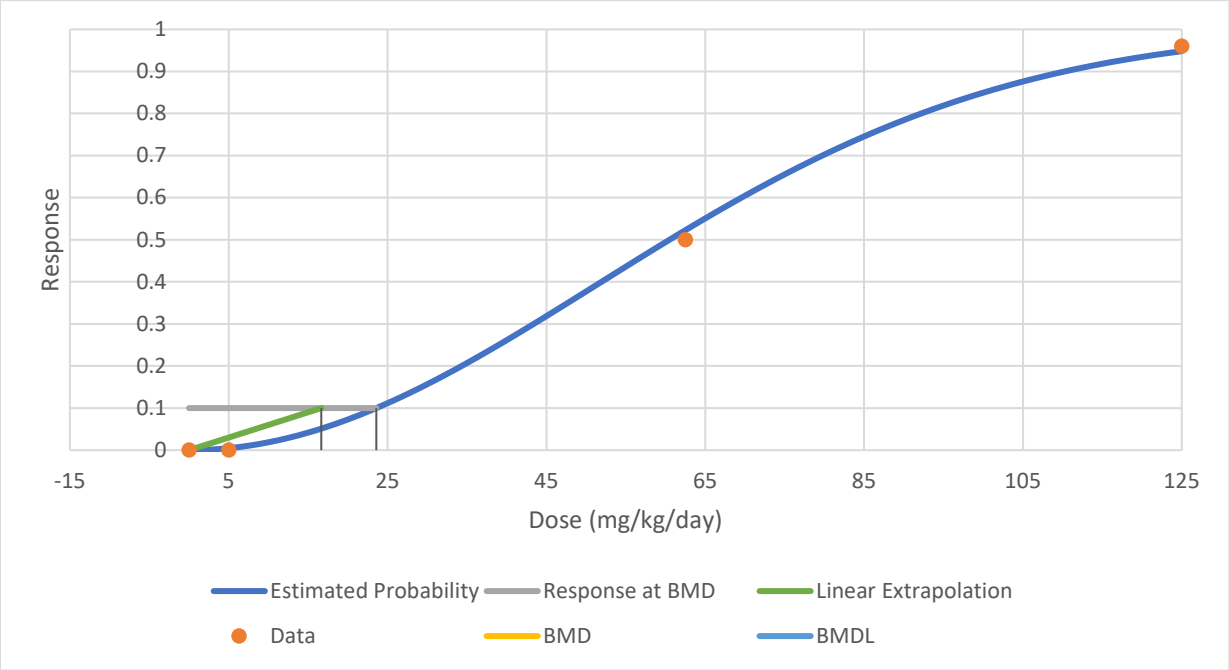
Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Scaled residuals[b] Dose below BMD	Dose above BMD	Overall largest	AIC	BMD_{10} (mg/kg/d)	$BMDL_{10}$ (mg/kg/d)
Gamma[c]	1	0.0014	0.97	-0.0368	0.0035	-0.0368	92.112	33.25	20.70
LogLogistic	1	0.0005	0.98	-0.0216	0.0006	-0.0216	92.110	38.71	27.32
Multistage (3-degree)[d]	1	0.1893	0.66	-0.423	0.0859	-0.423	92.477	26.11	16.00
Multistage (2-degree)[d,e]	**3**	**0.4844**	**0.92**	**-0.487**	**-0.323**	**-0.487**	**88.841**	**23.59**	**16.66**
Multistage (1-degree)[d]	3	11.342	0.01						
Weibull[c]	**1**	0.1275	0.72	-0.001	0.047	0.047	92.345	27.53	17.95
Dichotomous Hill[f]	0	0.0005	ND						
Logistic	2	3.7024	0.16	-0.8699	0.7077	-1.382	94.514	35.19	27.68
LogProbit[g]	1	1.5×10^{-6}	1.00	-0.0009	8.9×10^{-6}	8.9×10^{-6}	92.109	37.63	26.33
Probit	2	3.1532	0.21	-0.8026	0.9576	-1.093	94.007	32.63	25.34

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Slope restricted to ≥1.
[d]Power restricted to ≥1.
[e]Among models providing adequate fit to the data, $BMDL_{10}$ values varied by <3-fold; therefore, the model with the lowest AIC was selected as the best-fitting model (2-degree Multistage).
[f]Invalid degrees of freedom and χ^2 values.
[g]Betas restricted to ≥0.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure dose associated with 10% extra risk); DF = degrees of freedom; ND = not determined (degrees of freedom = 0; model saturated; goodness-of-fit not calculated)

The model providing the best fit (lowest AIC) is the Multistage (2-degree) model, which defined a BMD_{10} of 23.59 mg 2,4-D/kg/day and a $BMDL_{10}$ of 16.66 mg 2,4-D/kg/day. The fit of the multistage (2-degree) model is presented in Figure A-1.

Figure A-1. Fit of Multistage (2-Degree) Model for Degeneration/Regeneration in Descending Limb of Proximal Tubules of Male B6C3F1 Mice Administered 2,4-D in the Food for 2 Years



Calculations

Intermittent Exposure: Not applicable

Uncertainty Factor: The $BMDL_{10}$ of 16.66 mg/kg/day was divided by a total uncertainty factor of 100:

- 10 for extrapolation from animals to humans
- 10 for human variability

MRL = $BMDL_{10}$ ÷ uncertainty factors
MRL = 16.66 mg/kg/day ÷ (10 x 10) = 0.2 mg/kg/day

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Kidney lesions (degeneration of the descending portion of proximal convoluted tubules) were reported in male and female rats receiving 2,4-D from food at 75 mg/kg/day for 12 months (interim sacrifice in a 2-year study); the NOAEL was 5 mg/kg/day (Charles et al. 1996a; EPA 1996a). Both 12-month interim and 2-year terminal sacrifice among male and female B6C3F1 mice revealed kidney lesions in the descending portion of proximal convoluted tubules (degeneration in males and hypercellularity in females) at a dose level of 62.5 and 150 mg/kg/day for males and females, respectively, at both 12-month interim and 2-year terminal sacrifice (Charles et al. 1996a; EPA 1996b). The NOAEL in the mouse study was 5 mg/kg/day for both sexes. Increased relative kidney weight and increased incidence of slight degeneration of proximal tubules in outer zone of medulla were reported among male Sprague-Dawley rats receiving 2,4-D from food for up to 12 weeks at 45.3 mg/kg/day; the corresponding NOAEL was 16.6 mg/kg/day (Marty et al. 2013).

Agency Contacts (Chemical Managers): Obaid Faroon

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR 2,4-D

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to 2,4-D.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for 2,4-D. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of 2,4-D have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of 2,4-D are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects
Other noncancer effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for 2,4-D released for public comment in 2017; thus, the literature search was restricted to studies published between February 2014 and January 2018. The following main databases were searched in January 2018:

- PubMed
- National Library of Medicine's TOXLINE
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for 2,4-D. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures

and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to 2,4-D were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings

Database search date	Query string
PubMed	
01/2018	(("2,4-Dichlorophenoxyacetic Acid"[mh] OR 94-75-7[rn] OR 14214-89-2[rn] OR 2307-55-3[rn] OR 2702-72-9[rn] OR 3766-27-6[rn]) AND (2014/02/01 : 3000[dp] OR 2015/02/01 : 3000[mhda])) OR ((("(2,4-Dichlorophenoxy)acetic acid"[tw] OR "(2,4-dichlorophenoxy)-Acetic acid "[tw] OR "(2,4-Dichlorophenyloxy)acetic acid"[tw] OR "2-(2,4-Dichlorophenoxy)acetic acid"[tw] OR "2-(2,4-dichlorophenoxy)-Acetic acid"[tw] OR "2,4-D"[tw] OR "2,4-Dichlorophenoxyacetic acid"[tw] OR "2,4-dichloro-Phenoxyacetic acid"[tw] OR "2,4-Dichlorophenoxyethanoic acid"[tw] OR "2,4-PA"[tw] OR "Acme LV 4"[tw] OR "Acme LV 6"[tw] OR "Agricorn D"[tw] OR "Agrion"[tw] OR "Agrotect"[tw] OR "Aminopielik 50SL"[tw] OR "Aminopielik 720"[tw] OR "Ammonium 2,4-dichlorophenoxyacetate"[tw] OR "Amoxone"[tw] OR "Basalcoat"[tw] OR "BH 2,4-D"[tw] OR "Brush-rhap"[tw] OR "B-Selektonon"[tw] OR "Butoxy-D 3: 1 Liquid emulsifiable Brushkiller LV96"[tw] OR "Chipco turf herbicide D"[tw] OR "Chloroxone"[tw] OR "Citrus fix"[tw] OR "Crop rider"[tw] OR "Croprider"[tw] OR "Debroussaillant 600"[tw] OR "Ded-Weed LV-69"[tw] OR "Deherban"[tw] OR "De-Pester Ded-Weed LV-2"[tw] OR "Desormone"[tw] OR "Dezormon"[tw] OR "Dichlordon sodium"[tw] OR "Dichlorophenoxyacetic acid"[tw] OR "Diclordon"[tw] OR "Diconirt"[tw] OR "Diconirt D"[tw] OR "Dicopur"[tw] OR "Dikonirt"[tw] OR "Dikonirt D"[tw] OR "Dormon"[tw] OR "Dormone"[tw] OR "Emulsamine"[tw] OR "ENT 8,538"[tw] OR "Envert DT"[tw] OR "Esteron 44 weed killer"[tw] OR "Esteron 76 BE"[tw] OR "Estone"[tw] OR "Fernesta"[tw] OR "Fernimine"[tw] OR "Fernoxene"[tw] OR "Fernoxone"[tw] OR "Ferxone"[tw] OR "Foredex 75"[tw] OR "Green Cross Weed-No-More 80"[tw] OR "Herbidal"[tw] OR "Hivol-44"[tw] OR "HM 2010"[tw] OR "Hormit"[tw] OR "Huragan"[tw] OR "Invesamina 480SL"[tw] OR "Ipaner"[tw] OR "Isadiamineyeom"[tw] OR "Kar D"[tw] OR "Lawn-keep"[tw] OR "Lithium 2,4-dichlorophenoxyacetate"[tw] OR "Macondray"[tw] OR "Macrondray"[tw] OR "Monosan"[tw] OR "Monosan herbi"[tw] OR "Mota Maskros"[tw] OR "Moxone"[tw] OR "Netagrone"[tw] OR "Netagrone 600"[tw] OR "NSC 2925"[tw] OR "Pennamine D"[tw] OR "Pielik"[tw] OR "Pielik E"[tw] OR "Planotox"[tw] OR "Plantgard"[tw] OR "Potassium (2,4-dichlorophenoxy)acetate"[tw] OR "Potassium 2,4-dichlorophenoxyacetate"[tw] OR "Profiamina"[tw] OR "Red Devil Dry Weed Killer"[tw] OR "R-H Weed Rhap 20"[tw] OR "Scott's 4-XD Weed Control"[tw] OR "Silvaprop 1"[tw] OR "Sodium (2,4-dichlorophenoxy)acetate"[tw] OR "Sodium 2,4-dichlorophenoxyacetate"[tw] OR "Sodium diclordon"[tw] OR "Solushan"[tw] OR "Spray-hormite"[tw] OR "Spritz-hormit"[tw] OR "Superormone concentre"[tw] OR "Taficide"[tw] OR "Tiller S"[tw] OR "Tornado DF"[tw] OR "Tributon"[tw] OR "U 46D"[tw] OR "U 46DP"[tw] OR "U-46-D-Fluid"[tw] OR "U-5043"[tw] OR "Vergemaster"[tw] OR "Verton 2D"[tw] OR "Verton 38"[tw] OR "Vidon 638"[tw] OR "Visko-rhap low drift herbicides"[tw] OR "Visko-rhap low volatile 4l"[tw] OR "Weed TOX"[tw] OR "Weed-Ag-Bar"[tw] OR "Weedatul"[tw] OR "Weed-B-gon"[tw] OR "Weedez Wonder BAR"[tw] OR "Weedone"[tw] OR "Weed-rhap"[tw] OR "Weed-Rhap A-4"[tw] OR "Weed-Rhap B-266"[tw] OR "Weed-Rhap B-4"[tw] OR "Weed-

Table B-2. Database Query Strings

Database search date	Query string
	Rhap I-3.34"[tw] OR "Weed-Rhap LV-4-0"[tw] OR "Weedtrol"[tw]) AND (2014/02/01 : 3000[dp] OR 2015/02/01 : 3000[crdat] OR 2015/02/01 : 3000[edat])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR "toxicokinetics"[mh:noexp])) OR ((("(2,4-Dichlorophenoxy)acetic acid"[tw] OR "(2,4-dichlorophenoxy)-Acetic acid "[tw] OR "(2,4-Dichlorophenyloxy)acetic acid"[tw] OR "2-(2,4-Dichlorophenoxy)acetic acid"[tw] OR "2-(2,4-dichlorophenoxy)-Acetic acid"[tw] OR "2,4-D"[tw] OR "2,4-Dichlorophenoxyacetic acid"[tw] OR "2,4-dichloro-Phenoxyacetic acid"[tw] OR "2,4-Dichlorophenoxyethanoic acid"[tw] OR "2,4-PA"[tw] OR "Acme LV 4"[tw] OR "Acme LV 6"[tw] OR "Agricorn D"[tw] OR "Agrion"[tw] OR "Agrotect"[tw] OR "Aminopielik 50SL"[tw] OR "Aminopielik 720"[tw] OR "Ammonium 2,4-dichlorophenoxyacetate"[tw] OR "Amoxone"[tw] OR "Basalcoat"[tw] OR "BH 2,4-D"[tw] OR "Brush-rhap"[tw] OR "B-Selektonon"[tw] OR "Butoxy-D 3: 1 Liquid emulsifiable Brushkiller LV96"[tw] OR "Chipco turf herbicide D"[tw] OR "Chloroxone"[tw] OR "Citrus fix"[tw] OR "Crop rider"[tw] OR "Croprider"[tw] OR "Debroussaillant 600"[tw] OR "Ded-Weed LV-69"[tw] OR "Deherban"[tw] OR "De-Pester Ded-Weed LV-2"[tw] OR "Desormone"[tw] OR "Dezormon"[tw] OR "Dichlordon sodium"[tw] OR "Dichlorophenoxyacetic acid"[tw] OR "Diclordon"[tw] OR "Diconirt"[tw] OR "Diconirt D"[tw] OR "Dicopur"[tw] OR "Dikonirt"[tw] OR "Dikonirt D"[tw] OR "Dormon"[tw] OR "Dormone"[tw] OR "Emulsamine"[tw] OR "ENT 8,538"[tw] OR "Envert DT"[tw] OR "Esteron 44 weed killer"[tw] OR "Esteron 76 BE"[tw] OR "Estone"[tw] OR "Fernesta"[tw] OR "Fernimine"[tw] OR "Fernoxene"[tw] OR "Fernoxone"[tw] OR "Ferxone"[tw] OR "Foredex 75"[tw] OR "Green Cross Weed-No-More 80"[tw] OR "Herbidal"[tw] OR "Hivol-44"[tw] OR "HM 2010"[tw] OR "Hormit"[tw] OR "Huragan"[tw] OR "Invesamina 480SL"[tw] OR "Ipaner"[tw] OR "Isadiamineyeom"[tw] OR "Kar D"[tw] OR "Lawn-keep"[tw] OR "Lithium 2,4-dichlorophenoxyacetate"[tw] OR "Macondray"[tw] OR "Macrondray"[tw] OR "Monosan"[tw] OR "Monosan herbi"[tw] OR "Mota Maskros"[tw] OR "Moxone"[tw] OR "Netagrone"[tw] OR "Netagrone 600"[tw] OR "NSC 2925"[tw] OR "Pennamine D"[tw] OR "Pielik"[tw] OR "Pielik E"[tw] OR "Planotox"[tw] OR "Plantgard"[tw] OR "Potassium (2,4-dichlorophenoxy)acetate"[tw] OR "Potassium 2,4-dichlorophenoxyacetate"[tw] OR "Profiamina"[tw] OR "Red Devil Dry Weed Killer"[tw] OR "R-H Weed Rhap 20"[tw] OR "Scott's 4-XD Weed Control"[tw] OR "Silvaprop 1"[tw] OR "Sodium (2,4-dichlorophenoxy)acetate"[tw] OR "Sodium 2,4-dichlorophenoxyacetate"[tw] OR "Sodium diclordon"[tw] OR "Solushan"[tw] OR "Spray-hormite"[tw] OR "Spritz-hormit"[tw] OR "Superormone concentre"[tw] OR "Taficide"[tw] OR "Tiller S"[tw] OR "Tornado DF"[tw] OR "Tributon"[tw] OR "U 46D"[tw] OR "U 46DP"[tw] OR "U-46-D-Fluid"[tw] OR "U-5043"[tw] OR "Vergemaster"[tw] OR "Verton 2D"[tw] OR "Verton 38"[tw] OR "Vidon 638"[tw] OR "Visko-rhap low drift herbicides"[tw] OR "Visko-rhap low volatile 4l"[tw] OR "Weed TOX"[tw] OR "Weed-Ag-Bar"[tw] OR "Weedatul"[tw] OR "Weed-B-

Table B-2. Database Query Strings

Database search date	Query string
	gon"[tw] OR "Weedez Wonder BAR"[tw] OR "Weedone"[tw] OR "Weed-rhap"[tw] OR "Weed-Rhap A-4"[tw] OR "Weed-Rhap B-266"[tw] OR "Weed-Rhap B-4"[tw] OR "Weed-Rhap I-3.34"[tw] OR "Weed-Rhap LV-4-0"[tw] OR "Weedtrol"[tw]) AND (2014/02/01 : 3000[dp] OR 2015/02/01 : 3000[crdat] OR 2015/02/01 : 3000[edat])) NOT medline[sb])
	"2,4-Dichlorophenoxyacetic Acid"[mh] AND "Adverse Outcome Pathways"[mh]
	("5742-19-8"[rn] OR "2008-39-1"[rn] OR "5742-17-6"[rn] OR "18584-79-7"[rn] OR "1929-73-3"[rn] OR "1928-43-4"[rn] OR "94-11-1"[rn] OR "2,4-D amine"[nm] OR "butoxyethanol ester of 2,4-dichlorophenoxyacetic acid"[nm] OR "2-ethylhexyl 2,4-dichlorophenoxyacetate"[nm]) OR "2,4-D Bis(2-hydroxyethyl)ammonium"[tw] OR "2,4-D Diethanolamine"[tw] OR "2,4-D Diethanolamine salt"[tw] OR "2,4-D-Bis(2-hydroxyethyl)ammonium"[tw] OR "2,4-D-diolamine"[tw] OR "2,4-Dichlorophenoxyacetic acid diethanolamine salt"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2,2'-iminobis(ethanol) (1:1)"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, diethanolamine salt"[tw] OR "Bis(2-hydroxyethyl)ammonium 2,4-dichlorophenoxyacetate"[tw] OR "Diethanolamine 2,4-dichlorophenoxyacetate"[tw] OR "Diethanolamine salt of 2,4-dichlorophenoxyacetic acid solution"[tw] OR "(2,4-Dichlorophenoxy)acetic acid compd. with isopropylamine"[tw] OR "(2,4-Dichlorophenoxy)acetic acid isopropylamine salt"[tw] OR "2,4-D isopropylamine"[tw] OR "2,4-D isopropylamine salt"[tw] OR "2,4-D-isopropylammonium"[tw] OR "2-Propanamine, (2,4-dichlorophenoxy)acetate"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2-propanamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with isopropylamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropylamine salt"[tw] OR "Isopropylamine 2,4-dichlorophenoxyacetate"[tw] OR "Isopropylamine, compd. with (2,4-dichlorophenoxy)acetic acid"[tw] OR "(2,4-Dichlorophenoxy)acetic acid compd. with 1,1',1-nitrilotris[2-propanol]"[tw] OR "2,4-D Triisopropanolamine"[tw] OR "2,4-D triisopropanolamine salt"[tw] OR "2,4-D triisopropanolamine sodium salt"[tw] OR "2,4-D triisopropanolammonium salt"[tw] OR "2,4-D, triisopropanolamine salt"[tw] OR "2,4-D-tris(2-hydroxypropyl)ammonium"[tw] OR "2,4-D-Trisopropyl salt"[tw] OR "2,4-Dichlorophenoxyacetic acid triisopropanolamine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid, triisopropanolamine salt solution"[tw] OR "2-Propanol, 1,1',1"-nitrilotri-, (2,4-dichlorophenoxy)acetate (salt)"[tw] OR "2-Propanol, 1,1',1"-nitrilotris-, (2,4-dichlorophenoxy)acetate (salt)"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1"-nitrilotri-2-propanol"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1"-nitrilotris(2-propanol)"[tw] OR "Triisopropanolamine 2,4-dichlorophenoxyacetate"[tw] OR "(2,4-Dichlorophenoxy)acetic acid, 1-methylethyl ester"[tw] OR "2,4-D ester"[tw] OR "2,4-D esters"[tw] OR "2,4-D Isopropyl ester"[tw] OR "2,4-D, isopropyl ester"[tw] OR "2,4-D-ester"[tw] OR "2,4-D-Isopropyl"[tw] OR "2,4-Dichlorophenoxyacetic acid ester"[tw] OR "2,4-Dichlorophenoxyacetic acid, 1-methylethyl ester"[tw] OR "2,4-Dichlorophenoxyacetic acid, isopropyl ester"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, 1-methylethyl ester"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropyl ester"[tw] OR "Amchem Weed Killer 650"[tw] OR "Barber's Weed Killer (Ester Formulation)"[tw] OR "Bridgeport Spot Weed Killer"[tw] OR "Chemical Insecticide's Isopropyl Ester of 2,4-D Liquid Concentrate"[tw] OR "Crop Rider 3-34D-2"[tw] OR "Crop Rider 3.34D"[tw] OR "Esteron 44"[tw] OR "Isopropyl (2,4-dichlorophenoxy)acetate"[tw] OR "Isopropyl 2,4-D ester"[tw] OR "Isopropyl 2,4-dichlorophenoxyacetate"[tw] OR "Isopropylester kyseliny 2,4-dichlorfenoxyoctove"[tw] OR "Isopropylester kyseliny 2,4-dichlorfenoxyoctove [Czech]"[tw] OR "Monsanto 2,4-D Isopropyl Ester"[tw] OR "Niagara Estasol"[tw] OR "Parsons 2,4-D Weed Killer Isopropyl Ester"[tw] OR "Swift's Gold Bear 44 Ester"[tw] OR "Weedone 128"[tw]
	("(2,4-Dichlorophenoxy)acetic acid dimethylamine"[tw] OR "(2,4-Dichlorophenoxy)acetic acid dimethylamine salt"[tw] OR "2,4-D amine"[tw] OR "2,4-D amine salt"[tw] OR "2,4-D

Table B-2. Database Query Strings

Database search date	Query string
	dimethylamine"[tw] OR "2,4-D dimethylamine salt"[tw] OR "2,4-D DMA"[tw] OR "2,4-D N,N-dimethylamine"[tw] OR "2,4-D, alkanolamine salt"[tw] OR "2,4-D-dimethylammonium"[tw] OR "2,4-Diamin SL"[tw] OR "2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine"[tw] OR "2,4-Dichlorophenoxy)acetic acid dimethylamine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid dimethylamine"[tw] OR "2,4-Dichlorophenoxyacetic acid dimethylamine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid, dimethyl amine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid, dimethylamine salt solution"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with dimethylamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with N-methylmethanamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with dimethylamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with N-methylmethanamine"[tw] OR "Dimethylamine 2,4-dichlorophenoxyacetate"[tw] OR "Dimethylamine salt of 2,4-dichlorophenoxyacetic acid solution"[tw] OR "Dimethylamine, (2,4-dichlorophenoxy)acetate"[tw] OR "Dimethylammonium 2,4-dichlorophenoxyacetate"[tw] OR "Methanamine, N-methyl-, (2,4-dichlorophenoxy)acetate"[tw] OR "N-Methylmethanamine (2,4-dichlorophenoxy)acetate"[tw] OR "N-Methylmethanamine 2,4-dichlorophenoxyacetate"[tw] OR "Alkano amine salt of 2,4-D"[tw] OR "Amicide"[tw] OR "Amine-2,4-D"[tw] OR "Aminol"[tw] OR "Aminopielek 720"[tw] OR "Aminopielik 600SL"[tw] OR "Aminoprelik 39"[tw] OR "Amisol"[tw] OR "Banvel 3 Liquid Herbicide"[tw] OR "Banvel-720"[tw] OR "Barber's Weed Killer"[tw] OR "Best 4 Servis Brand Lawn Weed Killer"[tw] OR "Bladex G"[tw] OR "Blitz 64"[tw] OR "Brabant 2,4-D amine"[tw] OR "Chipman 2,4-D Amine No. 4"[tw] OR "Chipman Lawn Weedkiller"[tw] OR "Chipman's 2,4-D amine No. 4"[tw] OR "Clean Crop 2,4-D Amine 500"[tw] OR "Co-op Premium Lawn Weed Killer"[tw] OR "D 50 (pesticide)"[tw] OR "Ded-Weed Sulv"[tw] OR "Desormone"[tw] OR "Diamond Shamrock Amine 6D"[tw] OR "Dikamin D"[tw] OR "DMA 4"[tw] OR "DMA 6"[tw] OR "DMA-2,4-D"[tw] OR "Dma-4"[tw] OR "Dow DMA-4"[tw] OR "Dow Formula 40"[tw] OR "Du Pont Lawn Weed Killer"[tw] OR "Du Pont Turf Food With Weed Killer"[tw] OR "Du Pont Weed Killer No. 2"[tw] OR "Farmco D 50"[tw] OR "Farmco D-50"[tw] OR "Floro Tox 2,4-D Amine Weed Killer"[tw] OR "Formula 40"[tw] OR "FS Amine 400 Weed Killer"[tw] OR "Green Cross Killex Spot Weeder Pressurized Spray"[tw] OR "Green Cross Poison Ivy Killer"[tw] OR "Herbitex"[tw] OR "Hormin"[tw] OR "Liquid Clearit Vegetation Killer"[tw] OR "Liquid Wonder Weeder"[tw] OR "Manco Kill-Weed"[tw] OR "Marquette Herbitex Plus"[tw] OR "Mecoturf Plus 2,4-D Liquid Weedkiller"[tw] OR "Monosan"[tw] OR "Monsanto 2,4-D Amine"[tw] OR "Morselect"[tw] OR "Norkem 40t"[tw] OR "Ortho Super Weed-B-Gon Spray"[tw] OR "Pacific Cooperatives P 2,4-D Amine Weed Killer"[tw] OR "Parsons 2,4-D Weed Killer"[tw] OR "Parsons 2,4-D Weed Killer No. 40"[tw] OR "Phordene"[tw] OR "Reed amine 400"[tw] OR "Shirweed 500"[tw] OR "Spraygraze"[tw] OR "Spritz-Hormin"[tw] OR "Sure Death 2,4-D Amine Weedkiller"[tw] OR "Techne 2,4-D Amine Weed Killer"[tw] OR "U 46D Fluid"[tw] OR "U-46 D-Fluid"[tw] OR "Vigoro Dandelions Killer"[tw] OR "Weed-Rhap A-4D"[tw] OR "Weedar 64"[tw] OR "Weedar 96"[tw] OR "Weedkiller D"[tw] OR "Wilbur-Ellis 2,4-D Amine 500"[tw] OR "Wilson's Multi-Weeder"[tw] OR "Zehrung 2,4-D Selective Amine Weed Killer"[tw] OR "(2,4-Dichlorophenoxy)acetic acid butoxyethyl ester"[tw] OR "(2,4-Dichlorophenoxy)acetic acid, 2-butoxyethyl ester"[tw] OR "2,4-D (BEE)"[tw] OR "2,4-D (BOEE)"[tw] OR "2,4-D 2-butoxyethyl ester"[tw] OR "2,4-D butoxyethanol"[tw] OR "2,4-D butoxyethanol ester"[tw] OR "2,4-D butoxyethyl ester"[tw] OR "2,4-D esters"[tw] OR "2,4-D isobutoxyethanol"[tw] OR "2,4-D, BEE"[tw] OR "2,4-D, butoxyethanol ester"[tw] OR "2,4-D, butoxyethyl ester"[tw] OR "2,4-D-(2-Butoxyethyl)"[tw] OR "2,4-D-BEE"[tw] OR "2,4-D-butotyl"[tw] OR "2,4-DBE"[tw] OR "2,4-DBEE"[tw] OR "2,4-Dichlorophenoxyacetic acid 2-Butoxyethyl ester"[tw] OR "2,4-Dichlorophenoxyacetic acid butoxyethanol ester"[tw] OR "2,4-Dichlorophenoxyacetic acid ethylene glycol butyl ether ester"[tw] OR "2,4-

Table B-2. Database Query Strings

Database search date	Query string
	Dichlorophenoxyacetic acid, butoxyethyl ester"[tw] OR "2,4-Dichlorophenoxyacetic acids"[tw] OR "2-Butoxyethyl 2,4-dichlorophenoxyacetate"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, butoxyethyl ester"[tw] OR "Aqua-Kleen"[tw] OR "BEE 2,4-D"[tw] OR "Bladex-B"[tw] OR "Brush killer 64"[tw] OR "Butoxy-D 3"[tw] OR "Butoxyethanol ester of (2,4-dichlorophenoxy)acetic acid"[tw] OR "Butoxyethanol ester of 2,4-dichlorophenoxyacetic acid"[tw] OR "Butoxyethyl 2,4-dichlorophenoxyacetate"[tw] OR "Butoxyethyl ester of 2,4-dichlorophenoxy acetic acid"[tw] OR "Esteron 99 Concentrate"[tw] OR "Lo-Estasol"[tw] OR "Planotox"[tw] OR "Silvaprop 1"[tw] OR "Weed-Rhap LV-4D"[tw] OR "Weedone 100 Emulsifiable"[tw] OR "Weedone 638"[tw] OR "Weedone LV 4"[tw] OR "Weedone LV-6"[tw] OR "Weedone LV4"[tw] OR "(2,4-Dichlorophenoxy)acetic acid 2-ethylhexyl ester"[tw] OR "2,4-D 2-Ethylhexyl ester"[tw] OR "2,4-D Ethylhexyl ester"[tw] OR "2,4-D-2-ethylhexyl"[tw] OR "2-Ethylhexyl (2,4-dichlorophenoxy)acetate"[tw] OR "2-Ethylhexyl 2,4-dichlorophenoxyacetate"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR "toxicokinetics"[mh:noexp])
	("(2,4-Dichlorophenoxy)acetic acid dimethylamine"[tw] OR "(2,4-Dichlorophenoxy)acetic acid dimethylamine salt"[tw] OR "2,4-D amine"[tw] OR "2,4-D amine salt"[tw] OR "2,4-D dimethylamine"[tw] OR "2,4-D dimethylamine salt"[tw] OR "2,4-D DMA"[tw] OR "2,4-D N,N-dimethylamine"[tw] OR "2,4-D, alkanolamine salt"[tw] OR "2,4-D-dimethylammonium"[tw] OR "2,4-Diamin SL"[tw] OR "2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine"[tw] OR "2,4-Dichlorophenoxy)acetic acid dimethylamine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid dimethylamine"[tw] OR "2,4-Dichlorophenoxyacetic acid dimethylamine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid, dimethyl amine salt"[tw] OR "2,4-Dichlorophenoxyacetic acid, dimethylamine salt solution"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with dimethylamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with N-methylmethanamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with dimethylamine"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with N-methylmethanamine"[tw] OR "Dimethylamine 2,4-dichlorophenoxyacetate"[tw] OR "Dimethylamine salt of 2,4-dichlorophenoxyacetic acid solution"[tw] OR "Dimethylamine, (2,4-dichlorophenoxy)acetate"[tw] OR "Dimethylammonium 2,4-dichlorophenoxyacetate"[tw] OR "Methanamine, N-methyl-, (2,4-dichlorophenoxy)acetate"[tw] OR "N-Methylmethanamine (2,4-dichlorophenoxy)acetate"[tw] OR "N-Methylmethanamine 2,4-dichlorophenoxyacetate"[tw] OR "Alkano amine salt of 2,4-D"[tw] OR "Amicide"[tw] OR "Amine-2,4-D"[tw] OR "Aminol"[tw] OR "Aminopielek 720"[tw] OR "Aminopielik 600SL"[tw] OR "Aminoprelik 39"[tw] OR "Amisol"[tw] OR "Banvel 3 Liquid Herbicide"[tw] OR "Banvel-720"[tw] OR

Table B-2. Database Query Strings

Database search date	Query string
	"Barber's Weed Killer"[tw] OR "Best 4 Servis Brand Lawn Weed Killer"[tw] OR "Bladex G"[tw] OR "Blitz 64"[tw] OR "Brabant 2,4-D amine"[tw] OR "Chipman 2,4-D Amine No. 4"[tw] OR "Chipman Lawn Weedkiller"[tw] OR "Chipman's 2,4-D amine No. 4"[tw] OR "Clean Crop 2,4-D Amine 500"[tw] OR "Co-op Premium Lawn Weed Killer"[tw] OR "D 50 (pesticide)"[tw] OR "Ded-Weed Sulv"[tw] OR "Desormone"[tw] OR "Diamond Shamrock Amine 6D"[tw] OR "Dikamin D"[tw] OR "DMA 4"[tw] OR "DMA 6"[tw] OR "DMA-2,4-D"[tw] OR "Dma-4"[tw] OR "Dow DMA-4"[tw] OR "Dow Formula 40"[tw] OR "Du Pont Lawn Weed Killer"[tw] OR "Du Pont Turf Food With Weed Killer"[tw] OR "Du Pont Weed Killer No. 2"[tw] OR "Farmco D 50"[tw] OR "Farmco D-50"[tw] OR "Floro Tox 2,4-D Amine Weed Killer"[tw] OR "Formula 40"[tw] OR "FS Amine 400 Weed Killer"[tw] OR "Green Cross Killex Spot Weeder Pressurized Spray"[tw] OR "Green Cross Poison Ivy Killer"[tw] OR "Herbitex"[tw] OR "Hormin"[tw] OR "Liquid Clearit Vegetation Killer"[tw] OR "Liquid Wonder Weeder"[tw] OR "Manco Kill-Weed"[tw] OR "Marquette Herbitex Plus"[tw] OR "Mecoturf Plus 2,4-D Liquid Weedkiller"[tw] OR "Monosan"[tw] OR "Monsanto 2,4-D Amine"[tw] OR "Morselect"[tw] OR "Norkem 40t"[tw] OR "Ortho Super Weed-B-Gon Spray"[tw] OR "Pacific Cooperatives P 2,4-D Amine Weed Killer"[tw] OR "Parsons 2,4-D Weed Killer"[tw] OR "Parsons 2,4-D Weed Killer No. 40"[tw] OR "Phordene"[tw] OR "Reed amine 400"[tw] OR "Shirweed 500"[tw] OR "Spraygraze"[tw] OR "Spritz-Hormin"[tw] OR "Sure Death 2,4-D Amine Weedkiller"[tw] OR "Techne 2,4-D Amine Weed Killer"[tw] OR "U 46D Fluid"[tw] OR "U-46 D-Fluid"[tw] OR "Vigoro Dandelions Killer"[tw] OR "Weed-Rhap A-4D"[tw] OR "Weedar 64"[tw] OR "Weedar 96"[tw] OR "Weedkiller D"[tw] OR "Wilbur-Ellis 2,4-D Amine 500"[tw] OR "Wilson's Multi-Weeder"[tw] OR "Zehrung 2,4-D Selective Amine Weed Killer"[tw] OR "(2,4-Dichlorophenoxy)acetic acid butoxyethyl ester"[tw] OR "(2,4-Dichlorophenoxy)acetic acid, 2-butoxyethyl ester"[tw] OR "2,4-D (BEE)"[tw] OR "2,4-D (BOEE)"[tw] OR "2,4-D 2-butoxyethyl ester"[tw] OR "2,4-D butoxyethanol"[tw] OR "2,4-D butoxyethanol ester"[tw] OR "2,4-D butoxyethyl ester"[tw] OR "2,4-D esters"[tw] OR "2,4-D isobutoxyethanol"[tw] OR "2,4-D, BEE"[tw] OR "2,4-D, butoxyethanol ester"[tw] OR "2,4-D, butoxyethyl ester"[tw] OR "2,4-D-(2-Butoxyethyl)"[tw] OR "2,4-D-BEE"[tw] OR "2,4-D-butotyl"[tw] OR "2,4-DBE"[tw] OR "2,4-DBEE"[tw] OR "2,4-Dichlorophenoxyacetic acid 2-Butoxyethyl ester"[tw] OR "2,4-Dichlorophenoxyacetic acid butoxyethanol ester"[tw] OR "2,4-Dichlorophenoxyacetic acid ethylene glycol butyl ether ester"[tw] OR "2,4-Dichlorophenoxyacetic acid, butoxyethyl ester"[tw] OR "2,4-Dichlorophenoxyacetic acids"[tw] OR "2-Butoxyethyl 2,4-dichlorophenoxyacetate"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, butoxyethyl ester"[tw] OR "Aqua-Kleen"[tw] OR "BEE 2,4-D"[tw] OR "Bladex-B"[tw] OR "Brush killer 64"[tw] OR "Butoxy-D 3"[tw] OR "Butoxyethanol ester of (2,4-dichlorophenoxy)acetic acid"[tw] OR "Butoxyethanol ester of 2,4-dichlorophenoxyacetic acid"[tw] OR "Butoxyethyl 2,4-dichlorophenoxyacetate"[tw] OR "Butoxyethyl ester of 2,4-dichlorophenoxy acetic acid"[tw] OR "Esteron 99 Concentrate"[tw] OR "Lo-Estasol"[tw] OR "Planotox"[tw] OR "Silvaprop 1"[tw] OR "Weed-Rhap LV-4D"[tw] OR "Weedone 100 Emulsifiable"[tw] OR "Weedone 638"[tw] OR "Weedone LV 4"[tw] OR "Weedone LV-6"[tw] OR "Weedone LV4"[tw] OR "(2,4-Dichlorophenoxy)acetic acid 2-ethylhexyl ester"[tw] OR "2,4-D 2-Ethylhexyl ester"[tw] OR "2,4-D Ethylhexyl ester"[tw] OR "2,4-D-2-ethylhexyl"[tw] OR "2-Ethylhexyl (2,4-dichlorophenoxy)acetate"[tw] OR "2-Ethylhexyl 2,4-dichlorophenoxyacetate"[tw] OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester"[tw]) NOT medline[sb]
Toxline	
01/2018	Limited 2014-present: "(2,4-Dichlorophenoxy)acetic acid" OR "(2,4-dichlorophenoxy)-Acetic acid " OR "(2,4-Dichlorophenyloxy)acetic acid" OR "2-(2,4-Dichlorophenoxy)acetic acid" OR "2-(2,4-

Table B-2. Database Query Strings

Database search date	Query string
	dichlorophenoxy)-Acetic acid" OR "2,4-D" OR "2,4-Dichlorophenoxyacetic acid" OR "2,4-dichloro-Phenoxyacetic acid" OR "2,4-Dichlorophenoxyethanoic acid" OR "2,4-PA" OR "Acme LV 4" OR "Acme LV 6" OR "Agricorn D"
	Limited 2014-present: "Agrion" OR "Agrotect" OR "Aminopielik 50SL" OR "Aminopielik 720" OR "Ammonium 2,4-dichlorophenoxyacetate" OR "Amoxone" OR "Basalcoat" OR "BH 2,4-D" OR "Brush-rhap" OR "B-Selektonon" OR "Butoxy-D 3: 1 Liquid emulsifiable Brushkiller LV96" OR "Chipco turf herbicide D" OR "Chloroxone" OR "Citrus fix" OR "Crop rider" OR "Croprider" OR "Debroussaillant 600"
	Limited 2014-present: "Ded-Weed LV-69" OR "Deherban" OR "De-Pester Ded-Weed LV-2" OR "Desormone" OR "Dezormon" OR "Dichlordon sodium" OR "Dichlorophenoxyacetic acid" OR "Diclordon" OR "Diconirt" OR "Diconirt D" OR "Dicopur" OR "Dikonirt" OR "Dikonirt D" OR "Dormon" OR "Dormone" OR "Emulsamine" OR "ENT 8,538" OR "Envert DT" OR "Esteron 44 weed killer" OR "Esteron 76 BE" OR "Estone" OR "Fernesta"
	Limited 2014-present: "Fernimine" OR "Fernoxene" OR "Fernoxone" OR "Ferxone" OR "Foredex 75" OR "Green Cross Weed-No-More 80" OR "Herbidal" OR "Hivol-44" OR "HM 2010" OR "Hormit" OR "Huragan" OR "Invesamina 480SL" OR "Ipaner" OR "Isadiamineyeom" OR "Kar D" OR "Lawn-keep" OR "Lithium 2,4-dichlorophenoxyacetate" OR "Macondray" OR "Macrondray" OR "Monosan" OR "Monosan herbi"
	Limited 2014-present: "Mota Maskros" OR "Moxone" OR "Netagrone" OR "Netagrone 600" OR "NSC 2925" OR "Pennamine D" OR "Pielik" OR "Pielik E" OR "Planotox" OR "Plantgard" OR "Potassium (2,4-dichlorophenoxy)acetate" OR "Potassium 2,4-dichlorophenoxyacetate" OR "Profiamina" OR "Red Devil Dry Weed Killer" OR "R-H Weed Rhap 20" OR "Scott's 4-XD Weed Control" OR "Silvaprop 1"
	Limited 2014-present: "Sodium (2,4-dichlorophenoxy)acetate" OR "Sodium 2,4-dichlorophenoxyacetate" OR "Sodium diclordon" OR "Solushan" OR "Spray-hormite" OR "Spritz-hormit" OR "Superormone concentre" OR "Taficide" OR "Tiller S" OR "Tornado DF" OR "Tributon" OR "U 46D" OR "U 46DP" OR "U-46-D-Fluid" OR "U-5043" OR "Vergemaster" OR "Verton 2D" OR "Verton 38" OR "Vidon 638"
	Limited 2014-present: "Visko-rhap low drift herbicides" OR "Visko-rhap low volatile 4l" OR "Weed TOX" OR "Weed-Ag-Bar" OR "Weedatul" OR "Weed-B-gon" OR "Weedez Wonder BAR" OR "Weedone" OR "Weed-rhap" OR "Weed-Rhap A-4" OR "Weed-Rhap B-266" OR "Weed-Rhap B-4" OR "Weed-Rhap I-3.34" OR "Weed-Rhap LV-4-0" OR "Weedtrol" OR 94-75-7[rn] OR 14214-89-2[rn] OR 2307-55-3[rn] OR 2702-72-9[rn] OR 3766-27-6[rn]
	"Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2,2'-iminobis(ethanol) (1:1)" OR "Acetic acid, (2,4-dichlorophenoxy)-, diethanolamine salt" OR "Bis(2-hydroxyethyl)ammonium 2,4-dichlorophenoxyacetate" OR "Diethanolamine 2,4-dichlorophenoxyacetate" OR "2,4-Diamin SL" OR "2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine" OR "2,4-Dichlorophenoxy)acetic acid dimethylamine salt"
	"Acetic acid, (2,4-dichlorophenoxy)-, cmpd with dimethylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with N-methylmethanamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with dimethylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-,

Table B-2. Database Query Strings

Database search date	Query string
	compd. with N-methylmethanamine" OR "Dimethylamine 2,4-dichlorophenoxyacetate" OR "Dimethylamine, (2,4-dichlorophenoxy)acetate"
	"Dimethylammonium 2,4-dichlorophenoxyacetate" OR "Methanamine, N-methyl-, (2,4-dichlorophenoxy)acetate" OR "N-Methylmethanamine (2,4-dichlorophenoxy)acetate" OR "N-Methylmethanamine 2,4-dichlorophenoxyacetate" OR "Alkano amine salt of 2,4-D" OR "Amicide" OR "Amine-2,4-D" OR "Aminol" OR "Aminopielek 720" OR "Aminopielik 600SL" OR "Aminoprelik 39"
	"Amisol" OR "Banvel 3 Liquid Herbicide" OR "Banvel-720" OR "Barber's Weed Killer" OR "Best 4 Servis Brand Lawn Weed Killer" OR "Bladex G" OR "Blitz 64" OR "Chipman Lawn Weedkiller" OR "Co-op Premium Lawn Weed Killer" OR "D 50 pesticide" OR "Ded-Weed Sulv" OR "Desormone" OR "Diamond Shamrock Amine 6D" OR "Dikamin D" OR "DMA 4" OR "DMA 6" OR "DMA-2,4-D" OR "Dma-4" OR "Dow DMA-4"
	"Dow Formula 40" OR "Du Pont Lawn Weed Killer" OR "Du Pont Turf Food With Weed Killer" OR "Du Pont Weed Killer No. 2" OR "Farmco D 50" OR "Farmco D-50" OR "Formula 40" OR "FS Amine 400 Weed Killer" OR "Green Cross Killex Spot Weeder Pressurized Spray" OR "Green Cross Poison Ivy Killer" OR "Herbitex" OR "Hormin" OR "Liquid Clearit Vegetation Killer" OR "Liquid Wonder Weeder" OR "Manco Kill-Weed"
	"Marquette Herbitex Plus" OR "Monosan" OR "Morselect" OR "Norkem 40t" OR "Ortho Super Weed-B-Gon Spray" OR "Phordene" OR "Reed amine 400" OR "Shirweed 500" OR "Spraygraze" OR "Spritz-Hormin" OR "Vigoro Dandelions Killer" OR "Weed-Rhap A-4D" OR "Weedar 64" OR "Weedar 96" OR "Weedkiller D" OR "Wilson's Multi-Weeder" OR "2-Propanamine, (2,4-dichlorophenoxy)acetate"
	"Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2-propanamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with isopropylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropylamine salt" OR "Isopropylamine 2,4-dichlorophenoxyacetate" OR "2-Propanol, 1,1',1"-nitrilotri-, (2,4-dichlorophenoxy)acetate (salt)" OR "2-Propanol, 1,1',1"-nitrilotris-, (2,4-dichlorophenoxy)acetate (salt)"
	"Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1"-nitrilotri-2-propanol" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1"-nitrilotris(2-propanol)" OR "Triisopropanolamine 2,4-dichlorophenoxyacetate" OR "2,4-DBE" OR "2,4-DBEE" OR "2,4-Dichlorophenoxyacetic acids" OR "2-Butoxyethyl 2,4-dichlorophenoxyacetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester"
	"Acetic acid, (2,4-dichlorophenoxy)-, butoxyethyl ester" OR "Aqua-Kleen" OR "BEE 2,4-D" OR "Bladex-B" OR "Brush killer 64" OR "Butoxy-D 3" OR "Butoxyethyl 2,4-dichlorophenoxyacetate" OR "Butoxyethyl ester of 2,4-dichlorophenoxy acetic acid" OR "Esteron 99 Concentrate" OR "Lo-Estasol" OR "Planotox" OR "Silvaprop 1" OR "Weed-Rhap LV-4D" OR "Weedone 100 Emulsifiable" OR "Weedone 638"
	"Weedone LV 4" OR "Weedone LV-6" OR "Weedone LV4" OR "2-Ethylhexyl (2,4-dichlorophenoxy)acetate" OR "2-Ethylhexyl 2,4-dichlorophenoxyacetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, 1-methylethyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropyl ester" OR "Amchem Weed Killer 650"
	"Barber's Weed Killer (Ester Formulation)" OR "Bridgeport Spot Weed Killer" OR "Crop Rider 3-34D-2" OR "Crop Rider 3.34D" OR "Esteron 44" OR "Isopropyl (2,4-dichlorophenoxy)acetate" OR "Isopropyl 2,4-dichlorophenoxyacetate" OR "Niagara Estasol" OR "Swift's Gold Bear 44 Ester" OR "Weedone 128" OR 5742-19-8[rn] OR 2008-39-1[rn] OR 5742-17-6[rn] OR 18584-79-7[rn] OR 1929-73-3[rn] OR 1928-43-4[rn] OR 94-11-1[rn]

Table B-2. Database Query Strings

Database search date	Query string
Toxcenter	
01/2018	FILE 'TOXCENTER' ENTERED AT 10:27:31 ON 29 JAN 2018 CHARGED TO COST=EH011.05.LB.02.05 L1 14208 SEA FILE=TOXCENTER 94-75-7 OR 14214-89-2 OR 2307-55-3 OR 2702-72-9 OR 3766-27-6 L2 12917 SEA FILE=TOXCENTER L1 NOT PATENT/DT L3 12905 SEA FILE=TOXCENTER L2 NOT TSCATS/FS L4 630 SEA FILE=TOXCENTER L3 AND ED>=20150201 L5 759 SEA FILE=TOXCENTER 5742-19-8 OR 2008-39-1 OR 5742-17-6 OR 18584-79-7 OR 1929-73-3 OR 1928-43-4 OR 94-11-1 L6 674 SEA FILE=TOXCENTER L5 NOT PATENT/DT L7 674 SEA FILE=TOXCENTER L6 NOT TSCATS/FS L8 431 SEA FILE=TOXCENTER L7 NOT L1 ACT TOXQUERY/Q --------- L9 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L10 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L11 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L12 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L13 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L14 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L15 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L16 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L17 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L18 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L19 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L20 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L21 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L22 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L23 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L24 QUE (ENDOCRIN? AND DISRUPT?) L25 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L26 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)

Table B-2. Database Query Strings

Database search date	Query string
	L27 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L28 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L29 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) L30 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?) L31 QUE (NEPHROTOX? OR HEPATOTOX?) L32 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?) L33 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?) L34 QUE L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33 L35 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?) L36 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE) L37 QUE L34 OR L35 OR L36 L38 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?) L39 QUE L37 OR L38 --------- L40 355 SEA FILE=TOXCENTER L4 AND L39 L41 324 SEA FILE=TOXCENTER L7 AND L39 L42 186 SEA FILE=TOXCENTER L41 NOT L1 L43 321 SEA FILE=TOXCENTER L41 NOT L40 D SCAN L40 D SCAN L43

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS[a]	
01/2018	Compounds searched: 94-75-7; 14214-89-2; 2307-55-3; 2702-72-9; 3766-27-6; 5742-19-8; 2008-39-1; 5742-17-6; 18584-79-7; 1929-73-3; 1928-43-4; 94-11-1
NTP	
01/2018	Limited to dates 2014-1/2018 or not dated and Reports & Publications only. Terms searched: "94-75-7" OR "14214-89-2" OR "2307-55-3" OR "2702-72-9" OR "3766-27-6" "5742-19-8" OR "2008-39-1" OR "5742-17-6" OR "18584-79-7" OR "1929-73-3" OR "1928-43-4" OR "94-11-1"

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"(2,4-Dichlorophenoxy)acetic acid" OR "(2,4-dichlorophenoxy)-Acetic acid " OR "(2,4-Dichlorophenyloxy)acetic acid" OR "2-(2,4-Dichlorophenoxy)acetic acid" OR "2-(2,4-dichlorophenoxy)-Acetic acid" OR "2,4-D" OR "2,4-Dichlorophenoxyacetic acid" OR "2,4-dichloro-Phenoxyacetic acid" OR "2,4-Dichlorophenoxyethanoic acid" OR "2,4-PA" OR "Acme LV 4" OR "Acme LV 6" OR "Agricorn D" OR "Agrion" OR "Agrotect" OR "Aminopielik 50SL" OR "Aminopielik 720" OR "Ammonium 2,4-dichlorophenoxyacetate" OR "Amoxone" OR "Basalcoat" OR "BH 2,4-D" OR "Brush-rhap" OR "B-Selektonon" OR "Butoxy-D 3: 1 Liquid emulsifiable Brushkiller LV96" OR "Chipco turf herbicide D" OR "Chloroxone" OR "Citrus fix" OR "Crop rider" OR "Croprider" OR "Debroussaillant 600" OR "Ded-Weed LV-69" OR "Deherban" OR "De-Pester Ded-Weed LV-2" OR "Desormone" OR "Dezormon" OR "Dichlordon sodium" OR "Dichlorophenoxyacetic acid" OR "Diclordon" OR "Diconirt" OR "Diconirt D" OR "Dicopur" OR "Dikonirt" OR "Dikonirt D" OR "Dormon" OR "Dormone" OR "Emulsamine" OR "ENT 8,538" OR "Envert DT" OR "Esteron 44 weed killer" OR "Esteron 76 BE" OR "Estone" OR "Fernesta" OR "Fernimine" OR "Fernoxene" OR "Fernoxone" OR "Ferxone" OR "Foredex 75" OR "Green Cross Weed-No-More 80" OR "Herbidal" OR "Hivol-44" OR "HM 2010"
	"Hormit" OR "Huragan" OR "Invesamina 480SL" OR "Ipaner" OR "Isadiamineyeom" OR "Kar D" OR "Lawn-keep" OR "Lithium 2,4-dichlorophenoxyacetate" OR "Macondray" OR "Macrondray" OR "Monosan" OR "Monosan herbi" OR "Mota Maskros" OR "Moxone" OR "Netagrone" OR "Netagrone 600" OR "NSC 2925" OR "Pennamine D" OR "Pielik" OR "Pielik E" OR "Planotox" OR "Plantgard" OR "Potassium (2,4-dichlorophenoxy)acetate" OR "Potassium 2,4-dichlorophenoxyacetate" OR "Profiamina" OR "Red Devil Dry Weed Killer" OR "R-H Weed Rhap 20" OR "Scott's 4-XD Weed Control" OR "Silvaprop 1" OR "Sodium (2,4-dichlorophenoxy)acetate" OR "Sodium 2,4-dichlorophenoxyacetate" OR "Sodium diclordon" OR "Solushan" OR "Spray-hormite" OR "Spritz-hormit" OR "Superormone concentre" OR "Taficide" OR "Tiller S" OR "Tornado DF" OR "Tributon" OR "U 46D" OR "U 46DP" OR "U-46-D-Fluid" OR "U-5043" OR "Vergemaster" OR "Verton 2D" OR "Verton 38" OR "Vidon 638" OR "Visko-rhap low drift herbicides" OR "Visko-rhap low volatile 4l" OR "Weed TOX" OR "Weed-Ag-Bar" OR "Weedatul" OR "Weed-B-gon" OR "Weedez Wonder BAR" OR "Weedone" OR "Weed-rhap" OR "Weed-Rhap A-4" OR "Weed-Rhap B-266" OR "Weed-Rhap B-4" OR "Weed-Rhap I-3.34" OR "Weed-Rhap LV-4-0" OR "Weedtrol"
	"Bis(2-hydroxyethyl)ammonium 2,4-dichlorophenoxyacetate" OR "Diethanolamine 2,4-dichlorophenoxyacetate" OR "2,4-Diamin SL" OR "2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine" OR "2,4-Dichlorophenoxy)acetic acid dimethylamine salt" OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with dimethylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with N-methylmethanamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with dimethylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with N-methylmethanamine" OR "Dimethylamine 2,4-dichlorophenoxyacetate" OR "Dimethylamine, (2,4-dichlorophenoxy)acetate" OR "Dimethylammonium 2,4-dichlorophenoxyacetate" OR "Methanamine, N-methyl-, (2,4-dichlorophenoxy)acetate" OR "N-Methylmethanamine (2,4-dichlorophenoxy)acetate" OR "N-Methylmethanamine 2,4-dichlorophenoxyacetate" OR "Alkano amine salt of 2,4-D" OR "Amicide" OR "Amine-2,4-D" OR "Aminol" OR "Aminopielek 720" OR "Aminopielik 600SL" OR "Aminoprelik 39"
	"Amisol" OR "Banvel 3 Liquid Herbicide" OR "Banvel-720" OR "Barber's Weed Killer" OR "Best 4 Servis Brand Lawn Weed Killer" OR "Bladex G" OR "Blitz 64" OR "Chipman Lawn Weedkiller" OR "Co-op Premium Lawn Weed Killer" OR "D 50

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	(pesticide)" OR "Ded-Weed Sulv" OR "Desormone" OR "Diamond Shamrock Amine 6D" OR "Dikamin D" OR "DMA 4" OR "DMA 6" OR "DMA-2,4-D" OR "Dma-4" OR "Dow DMA-4" OR "Dow Formula 40" OR "Du Pont Lawn Weed Killer" OR "Du Pont Turf Food With Weed Killer" OR "Du Pont Weed Killer No. 2" OR "Farmco D 50" OR "Farmco D-50" OR "FS Amine 400 Weed Killer" OR "Green Cross Killex Spot Weeder Pressurized Spray" OR "Green Cross Poison Ivy Killer" OR "Herbitex" OR "Hormin" OR "Liquid Clearit Vegetation Killer" OR "Liquid Wonder Weeder" OR "Manco Kill-Weed" OR "Marquette Herbitex Plus" OR "Monosan" OR "Morselect" OR "Norkem 40t" OR "Ortho Super Weed-B-Gon Spray" OR "Phordene" OR "Reed amine 400" OR "Shirweed 500" OR "Spraygraze" OR "Spritz-Hormin" OR "Vigoro Dandelions Killer" OR "Weed-Rhap A-4D" OR "Weedar 64" OR "Weedar 96" OR "Weedkiller D" OR "Wilson's Multi-Weeder" OR "2-Propanamine, (2,4-dichlorophenoxy)acetate"
	"Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2-propanamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with isopropylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropylamine salt" OR "Isopropylamine 2,4-dichlorophenoxyacetate" OR "2-Propanol, 1,1',1"-nitrilotri-, (2,4-dichlorophenoxy)acetate (salt)" OR "2-Propanol, 1,1',1"-nitrilotris-, (2,4-dichlorophenoxy)acetate (salt)" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1"-nitrilotri-2-propanol" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1"-nitrilotris(2-propanol)" OR "Triisopropanolamine 2,4-dichlorophenoxyacetate" OR "2,4-DBE" OR "2,4-DBEE" OR "2,4-Dichlorophenoxyacetic acids" OR "2-Butoxyethyl 2,4-dichlorophenoxyacetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, butoxyethyl ester" OR "Aqua-Kleen" OR "BEE 2,4-D" OR "Bladex-B" OR "Brush killer 64" OR "Butoxy-D 3" OR "Butoxyethyl 2,4-dichlorophenoxyacetate" OR "Butoxyethyl ester of 2,4-dichlorophenoxy acetic acid" OR "Esteron 99 Concentrate" OR "Lo-Estasol" OR "Planotox" OR "Silvaprop 1" OR "Weed-Rhap LV-4D" OR "Weedone 100 Emulsifiable" OR "Weedone 638"
	"Weedone LV 4" OR "Weedone LV-6" OR "Weedone LV4" OR "2-Ethylhexyl (2,4-dichlorophenoxy)acetate" OR "2-Ethylhexyl 2,4-dichlorophenoxyacetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, 1-methylethyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropyl ester" OR "Amchem Weed Killer 650" OR "Barber's Weed Killer (Ester Formulation)" OR "Bridgeport Spot Weed Killer" OR "Crop Rider 3-34D-2" OR "Crop Rider 3.34D" OR "Esteron 44" OR "Isopropyl (2,4-dichlorophenoxy)acetate" OR "Isopropyl 2,4-dichlorophenoxyacetate" OR "Niagara Estasol" OR "Swift's Gold Bear 44 Ester" OR "Weedone 128" OR "Acetic acid, (2,4-dichlorophenoxy)-, diethanolamine salt" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2,2'-iminobis(ethanol)"
NPIRS	
01/2018	Limited 2015-present. CASRNs searched: 94-75-7 OR 14214-89-2 OR 2307-55-3 OR 2702-72-9 OR 3766-27-6
Regulations.gov	
01/2018	CASRNs searched: 94-75-7; 14214-89-2; 2307-55-3; 2702-72-9; 3766-27-6; 5742-19-8; 2008-39-1; 5742-17-6; 18584-79-7; 1929-73-3; 1928-43-4; 94-11-1

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
NIH RePORTER	
05/2019	Limited to Active projects. Terms searched: "94-75-7" OR "14214-89-2" OR "2307-55-3" OR "2702-72-9" OR "3766-27-6" OR "(2,4-Dichlorophenoxy)acetic acid" OR "(2,4-dichlorophenoxy)-Acetic acid " OR "(2,4-Dichlorophenyloxy)acetic acid" OR "2-(2,4-Dichlorophenoxy)acetic acid" OR "2-(2,4-dichlorophenoxy)-Acetic acid" OR "2,4-Dichlorophenoxyacetic acid" OR "2,4-dichloro-Phenoxyacetic acid" OR "2,4-Dichlorophenoxyethanoic acid" OR "2,4-PA" OR "Ammonium 2,4-dichlorophenoxyacetate" OR "Dichlorophenoxyacetic acid" OR "Lithium 2,4-dichlorophenoxyacetate" OR "Potassium (2,4-dichlorophenoxy)acetate" OR "Potassium 2,4-dichlorophenoxyacetate" OR "Sodium (2,4-dichlorophenoxy)acetate" OR "Sodium 2,4-dichlorophenoxyacetate" "5742-19-8" OR "2008-39-1" OR "5742-17-6" OR "18584-79-7" OR "1929-73-3" OR "1928-43-4" OR "94-11-1" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2,2'-iminobis(ethanol) (1:1)" OR "Acetic acid, (2,4-dichlorophenoxy)-, diethanolamine salt" OR "Bis(2-hydroxyethyl)ammonium 2,4-dichlorophenoxyacetate" OR "Diethanolamine 2,4-dichlorophenoxyacetate" OR "2,4-Dichlorophenoxy)acetic acid compd. with N-methylmethanamine" OR "2,4-Dichlorophenoxy)acetic acid dimethylamine salt" OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with dimethylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, cmpd with N-methylmethanamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with dimethylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with N-methylmethanamine" OR "Dimethylamine 2,4-dichlorophenoxyacetate" OR "Dimethylamine, (2,4-dichlorophenoxy)acetate" OR "Dimethylammonium 2,4-dichlorophenoxyacetate" OR "Methanamine, N-methyl-, (2,4-dichlorophenoxy)acetate" OR "N-Methylmethanamine (2,4-dichlorophenoxy)acetate" OR "N-Methylmethanamine 2,4-dichlorophenoxyacetate" OR "2-Propanamine, (2,4-dichlorophenoxy)acetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 2-propanamine" "Acetic acid, (2,4-dichlorophenoxy)-, compd. with isopropylamine" OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropylamine salt" OR "Isopropylamine 2,4-dichlorophenoxyacetate" OR "2-Propanol, 1,1',1''-nitrilotri-, (2,4-dichlorophenoxy)acetate (salt)" OR "2-Propanol, 1,1',1''-nitrilotris-, (2,4-dichlorophenoxy)acetate (salt)" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1''-nitrilotri-2-propanol" OR "Acetic acid, (2,4-dichlorophenoxy)-, compd. with 1,1',1''-nitrilotris(2-propanol)" OR "Triisopropanolamine 2,4-dichlorophenoxyacetate" OR "2,4-DBE" OR "2,4-DBEE" OR "2,4-Dichlorophenoxyacetic acids" OR "2-Butoxyethyl 2,4-dichlorophenoxyacetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, butoxyethyl ester" OR "Butoxyethyl 2,4-dichlorophenoxyacetate" OR "Butoxyethyl ester of 2,4-dichlorophenoxy acetic acid" OR "2-Ethylhexyl (2,4-dichlorophenoxy)acetate" OR "2-Ethylhexyl 2,4-dichlorophenoxyacetate" OR "Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, 1-methylethyl ester" OR "Acetic acid, (2,4-dichlorophenoxy)-, isopropyl ester" OR "Isopropyl (2,4-dichlorophenoxy)acetate" OR "Isopropyl 2,4-dichlorophenoxyacetate"
Other	Identified throughout the assessment process

[a]Several versions of the TSCATS database were searched, as needed, by CASRN including TSCATS1 via Toxline (no date limit), TSCATS2 via https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm (date restricted by EPA receipt date), and TSCATS via CDAT (date restricted by 'Mail Received Date Range'), as well as google for recent TSCA submissions.

The 2018 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 794
- Number of records identified from other strategies: 36
- Total number of records to undergo literature screening: 830

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on 2,4-D:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

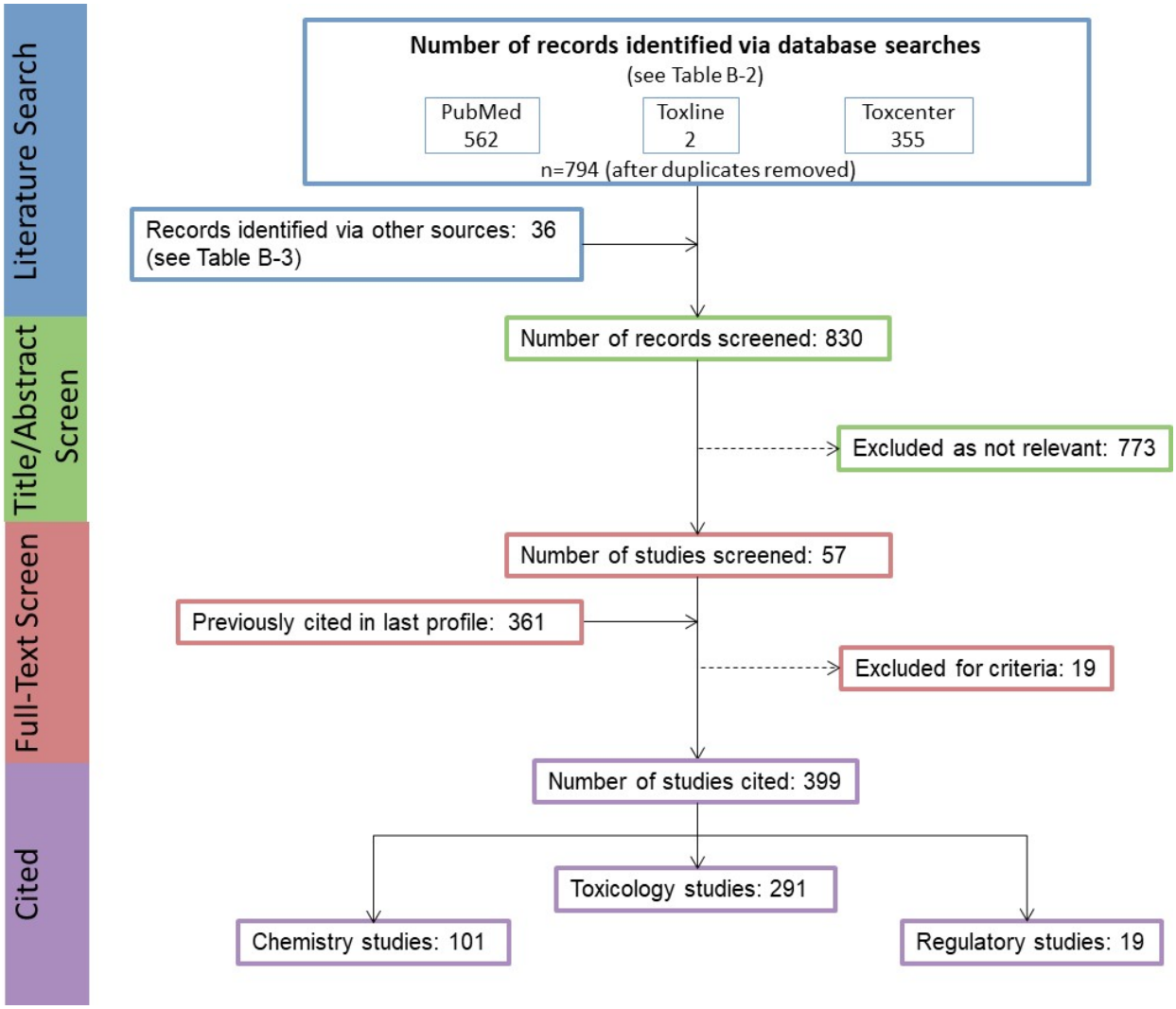
- Number of titles and abstracts screened: 830
- Number of studies considered relevant and moved to the next step: 57

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 57
- Number of studies cited in the pre-public draft of the toxicological profile: 361
- Total number of studies cited in the profile: 399

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. January 2018 Literature Search Results and Screen for 2,4-D



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX C

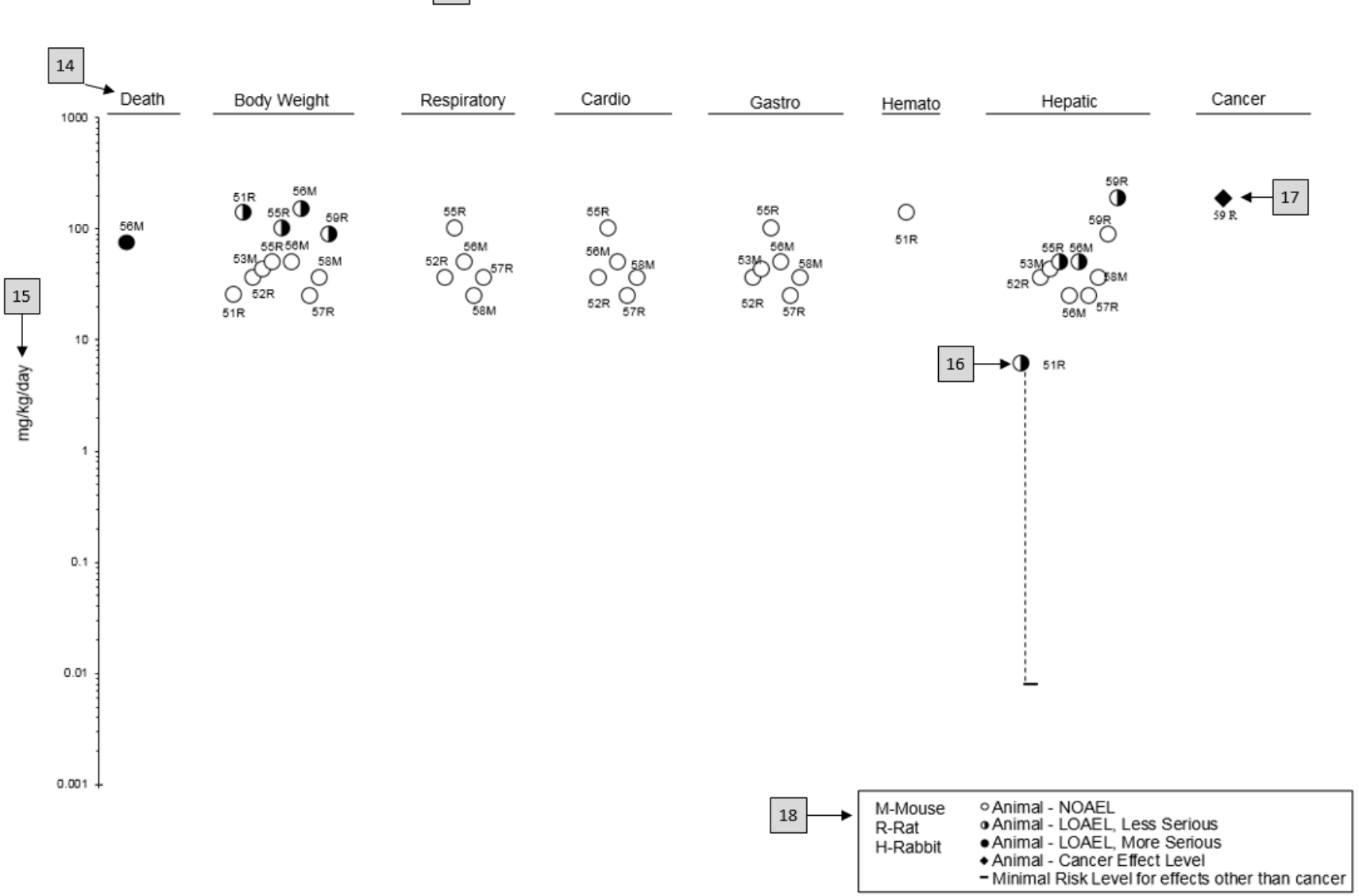
Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral

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
CHRONIC EXPOSURE									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

[a]The number corresponds to entries in Figure 2-x.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

APPENDIX C

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp). Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AHS	Agricultural Health Study
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration

FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram

NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture

USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result