3. HEALTH EFFECTS

3.1 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 end points 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. Therefore, studies of humans involved in these activities are summarized in Section 3.2.3, Dermal Exposure. 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.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies.

***DRAFT FOR PUBLIC COMMENT***
3. HEALTH EFFECTS

LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

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

### 3.2.1 Inhalation Exposure

Most of the information available regarding exposure to 2,4-D and health end points 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. Therefore, studies of humans involved in these activities are summarized in Section 3.2.3, Dermal Exposure. 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.

Information available regarding health effects in animals following inhalation exposure was limited to a report of deaths in rats and a 28-day inhalation study in rats that examined a wide range of end points (EPA 2008). The study also included observations during a recovery period.

### 3.2.1.1 Death

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 $\leq$1,000 mg/m$^3$ 2,4-D dusts 6 hours/day, 5 days/week for 28 days (EPA 2008).

### 3.2.1.2 Systemic Effects

The highest NOAEL values and all LOAEL values from the EPA (2008) study for systemic effects are recorded in Table 3-1 and plotted in Figure 3-1.

**Respiratory Effects.** Labored breathing was reported in rats exposed intermittently nose-only to 1,000 mg/m$^3$ 2,4-D dust in a 28-day inhalation study (EPA 2008). The effect was first seen on the 12$^{th}$ exposure; no such effect was seen in rats exposed to $\leq$300 mg/m$^3$ 2,4-D. 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$^3$ 2,4-D). 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.

**Cardiovascular Effects.** No gross or microscopic lesions were reported in the heart or thoracic aorta from rats intermittently exposed nose-only to $\leq$1,000 mg/m$^3$ 2,4-D dusts for 28 days (EPA 2008).

**Gastrointestinal Effects.** Intermittent nose-only exposure of rats to $\leq$1,000 mg/m$^3$ 2,4-D dusts for 28 days did not induce gross or microscopic lesions in the gastrointestinal tract, including the pancreas (EPA 2008).
### Table 3-1 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Inhalation

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency</th>
<th>System</th>
<th>NOAEL (mg/m³)</th>
<th>LOAEL Less Serious (mg/m³)</th>
<th>Serious (mg/m³)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat (Sprague-Dawley)</td>
<td>28 d 5 d/wk 6 hr/d</td>
<td>Resp</td>
<td>50</td>
<td>1000 (squamous epithelial hyperplasia and metaplasia in larynx)</td>
<td>1000 (labored breathing)</td>
<td>EPA 2008</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAELs are for histopathology of organs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cardio</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastro</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hemato</td>
<td>100 300</td>
<td>(20-26% decrease in reticulocytes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Musc/skel</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hepatic</td>
<td>100 300 F</td>
<td>(24% increased serum alkaline phosphatase)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Renal</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Endocr</td>
<td>1000</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Dermal</td>
<td>1000</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ocular</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bd Wt</td>
<td>300 F 1000 F</td>
<td>(11-13% reduced body weight during recovery)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metab</td>
<td>1000</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**INTERMEDIATE EXPOSURE**

**Systemic**

**Immuno/ Lymphoret**

2 Rat (Sprague-Dawley) 28 d 5 d/wk 6 hr/d 1000 EPA 2008 2,4-dichlorophenoxyacetic acid NOAEL is for histopathology of lymphoreticular tissues.
### Table 3-1 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Inhalation (continued)

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency</th>
<th>NOAEL (mg/m³)</th>
<th>Less Serious (mg/m³)</th>
<th>Serious (mg/m³)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
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<td>Neurological</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rat (Sprague-Dawley)</td>
<td>28 d 5 d/wk 6 hr/d</td>
<td>1000</td>
<td></td>
<td></td>
<td>EPA 2008</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of brain, spinal cord, and peripheral nerves.</td>
</tr>
<tr>
<td>Reproductive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EPA 2008</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs.</td>
</tr>
<tr>
<td>4</td>
<td>Rat (Sprague-Dawley)</td>
<td>28 d 5 d/wk 6 hr/d</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*a The number corresponds to entries in Figure 3-1.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-o bserved-adverse-effect level; Metab = metabolism; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)
Figure 3-1 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Inhalation

Intermediate (15-364 days)
3. HEALTH EFFECTS

**Hematological Effects.** Hematology tests conducted on male and female rats intermittently exposed nose-only to \( \geq 300 \text{ mg/m}^3 \) 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.

**Musculoskeletal Effects.** Intermittent nose-only exposure of rats to \( \leq 1,000 \text{ mg/m}^3 \) 2,4-D dusts for 28 days did not induce gross or microscopic lesions in bone or skeletal muscle (EPA 2008).

**Hepatic Effects.** 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 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.

**Renal Effects.** Intermittent nose-only exposure of rats to \( \leq 1,000 \text{ mg/m}^3 \) 2,4-D dusts 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.

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

**Dermal Effects.** Examination of the skin of rats exposed intermittently nose-only to \( \leq 1,000 \text{ mg/m}^3 \) 2,4-D dusts for 28 days did not show gross lesions (EPA 2008).
**Ocular Effects.** Ophthalmoscopic examination of the eyes from rats intermittently exposed nose-only to \( \leq 1,000 \text{ mg/m}^3 \) 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.

**Body Weight Effects.** 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 \( \leq 300 \text{ mg/m}^3 \) 2,4-D. In males, differences between exposed and control groups were either not statistically significant or were \( \leq 10\% \).

**Metabolic Effects.** Intermittent nose-only exposure of rats to \( \leq 1,000 \text{ mg/m}^3 \) 2,4-D dusts for 28 days did not significant alter serum electrolytes or glucose levels (EPA 2008).

**3.2.1.3 Immunological and Lymphoreticular Effects**

Significant increases in absolute and relative (to body weight and brain) spleen weight occurred in male rats intermittently exposed nose-only to \( \leq 1,000 \text{ mg/m}^3 \) 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 and are not listed in Table 3-1.

The exposure concentration of 1,000 mg/m\(^3\) is listed as a NOAEL for lymphoreticular effects in rats in Table 3-1 and plotted in Figure 3-1.

**3.2.1.4 Neurological Effects**

No treatment-related gross or microscopic alterations were reported in the brain, spinal cord, or peripheral nerves from rats intermittently exposed nose-only to \( \leq 1,000 \text{ mg/m}^3 \) 2,4-D dusts for 28 days (EPA 2008).

The NOAEL value for neurological effects in rats from EPA (2008) is recorded in Table 3-1 and plotted in Figure 3-1.
3.2.1.5 Reproductive Effects

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).

The NOAEL value for reproductive effects in rats from EPA (2008) is recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in animals following inhalation exposure to 2,4-D.

3.2.1.7 Cancer

No studies were located regarding cancer in humans or animals following inhalation exposure to 2,4-D.

3.2.2 Oral Exposure

As previously mentioned, 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. Therefore, studies of humans involved in these activities are summarized in Section 3.2.3, Dermal Exposure.

Information regarding oral exposure to 2,4-D in humans comes mainly from case reports of intentional or accidental ingestion of commercial herbicide formulations. Because most of these products also contained other ingredients that can be toxic (i.e., organic solvents, kerosene-like solvents or other herbicides), health outcomes observed following exposure cannot totally be attributed to 2,4-D. Additional information regarding clinical features of acute exposure to chlorophenoxy herbicides can be found in Bradberry et al. (2000).
3. HEALTH EFFECTS

3.2.2.1 Death

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.

Studies in rats have reported oral LD_{50} values between 600 and 800 mg/kg for 2,4-D (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. In a developmental study, repeated doses of 115 mg/kg 2,4-D decreased survival of pregnant rats (Chernoff et al. 1990). 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 Carslisle (1947) noted that some combination of some of these signs resembled myotonia congenita.

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.

LD$_{50}$ values and lethal doses are presented in Table 3-2 and plotted in Figure 3-2.

### 3.2.2.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

**Respiratory Effects.** 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).

With one exception, 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 to ≤125 mg 2,4-D/kg (Steiss et al. 1987) and in intermediate-duration studies in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), and dogs exposed to 7.5 mg 2,4-D/kg/day (Charles et al. 1996c).
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Duration</th>
<th>Frequency</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>LOAEL</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat (Sprague-Dawley)</td>
<td>10 d</td>
<td>1 x/d</td>
<td>Gd 6-15</td>
<td>115 F (decreased survival)</td>
<td>600 M (LD50)</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Rat (Sprague-Dawley)</td>
<td>once</td>
<td>(G)</td>
<td>600 M (LD50)</td>
<td>639 M (LD50)</td>
<td>666 (LD50)</td>
<td>764 F (LD50)</td>
<td>Elo et al. 1988</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rat (Fischer-344) (GO)</td>
<td>once</td>
<td>(GO)</td>
<td>639 M (LD50)</td>
<td>639 M (LD50)</td>
<td>500 (lethal dose)</td>
<td>Mattsson et al. 1997</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Rat (Fischer-344) (GO)</td>
<td>once</td>
<td>(GW)</td>
<td>666 (LD50)</td>
<td>666 (LD50)</td>
<td>500 (lethal dose)</td>
<td>Mattsson et al. 1997</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<tr>
<td>5</td>
<td>Rat (Fischer-344) (GO)</td>
<td>once</td>
<td>(GW)</td>
<td>375 (LD50)</td>
<td>375 (LD50)</td>
<td>500 (lethal dose)</td>
<td>Hill and Carlisle 1947</td>
<td>Sodium (2,4-dichlorophenoxy) acetate</td>
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<td>6</td>
<td>Mouse White</td>
<td>once</td>
<td>(GW)</td>
<td>1000 (LD50)</td>
<td>1000 (LD50)</td>
<td>1000 (LD50)</td>
<td>Hill and Carlisle 1947</td>
<td>Sodium (2,4-dichlorophenoxy) acetate</td>
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<td>7</td>
<td>Gn Pig (NS)</td>
<td>once</td>
<td>(GW)</td>
<td>1000 (LD50)</td>
<td>1000 (LD50)</td>
<td>1000 (LD50)</td>
<td>Drill and Hiratzka 1953</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>LOAEL</th>
<th>Less Serious (mg/kg/day)</th>
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<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tbody>
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<td>9</td>
<td>Rabbit</td>
<td>once (GW)</td>
<td></td>
<td></td>
<td></td>
<td>800 (LD50)</td>
<td></td>
<td>Hill and Carlisle 1947</td>
<td>Sodium (2,4-dichlorophenoxy) acetate</td>
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</tr>
<tr>
<td>10</td>
<td>Rat (Fischer-344)</td>
<td>10 d Gd 6-15 1 x/d (GW)</td>
<td>Bd Wt</td>
<td>75 F</td>
<td></td>
<td></td>
<td></td>
<td>Charles et al. 2001</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Rat (Sprague-Dawley)</td>
<td>10 d Gd 6-15 1 x/d (GO)</td>
<td>Bd Wt</td>
<td>115 M (decreased weight gain during treatment)</td>
<td></td>
<td></td>
<td></td>
<td>Chernoff et al. 1990</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td>12</td>
<td>Rat (Wistar)</td>
<td>9 d Gd 6-15 1 x/d (GW)</td>
<td>Bd Wt</td>
<td>50 F (weight loss during pregnancy)</td>
<td></td>
<td></td>
<td></td>
<td>Fofana et al. 2000</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<tr>
<td>13</td>
<td>Rat (Fischer-344) (GO)</td>
<td>once</td>
<td>Musc/skel</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td>Mattsson et al. 1997</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td></td>
<td></td>
<td></td>
<td>Endocr</td>
<td>250</td>
<td></td>
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<td>NOAEL is for histopathology of the pituitary, retina, and skeletal muscle tissue.</td>
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<td></td>
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<td>Ocular</td>
<td>250</td>
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<td></td>
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<td>Bd Wt</td>
<td>250</td>
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<tr>
<td>14</td>
<td>Rat (Sprague-Dawley)</td>
<td>Gd 6-15 10 d 1 x/d (GO)</td>
<td>Bd Wt</td>
<td>87.5 F</td>
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<td>Schwetz et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td>Key to Figure</td>
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<td>Exposure/Duration/Frequency (Route)</td>
<td>System</td>
<td>NOAEL (mg/kg/day)</td>
<td>LOAEL (mg/kg/day)</td>
<td>Reference</td>
<td>Chemical Form</td>
<td>Comments</td>
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<tr>
<td>15</td>
<td>Rat (Wistar)</td>
<td>6 d (F)</td>
<td>Endocr</td>
<td>15 F</td>
<td>(significant reduction in serum prolactin)</td>
<td>Sturtz et al. 2008</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td>16</td>
<td>Mouse (ICR)</td>
<td>10 d Gd 0-9 (W)</td>
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<td>100 F</td>
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<td>Dinamarca et al. 2007</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<tr>
<td>17</td>
<td>Dog (Beagle)</td>
<td>once (C)</td>
<td>Gastro</td>
<td>200 F</td>
<td>(vomiting and diarrhea)</td>
<td>Dickow et al. 2000</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td></td>
<td></td>
<td></td>
<td>Musc/skel</td>
<td>200 F</td>
<td>(insertional myotonia)</td>
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<td>Hepatic</td>
<td>200 F</td>
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<td>Renal</td>
<td>200 F</td>
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<td></td>
<td></td>
<td>Metab</td>
<td>200 F</td>
<td>(reduced serum calcium and potassium)</td>
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<td>18</td>
<td>Dog (Mongrel)</td>
<td>once (C)</td>
<td>Resp</td>
<td>125 F</td>
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<td></td>
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<td>Cardio</td>
<td>125 F</td>
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<td>Gastro</td>
<td>125 F</td>
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<td></td>
<td>Musc/skel</td>
<td>125 F</td>
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<td>Hepatic</td>
<td>125 F</td>
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<td>Renal</td>
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NOAELs are for organ histopathology.
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<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tbody>
<tr>
<td>19</td>
<td>Rabbit (New Zealand)</td>
<td>13 d Gd 6-18 1 x/d (GW)</td>
<td>Bd Wt</td>
<td>90 F</td>
<td></td>
<td></td>
<td>Charles et al. 2001</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
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<tr>
<td>20</td>
<td>Dog (Mongrel)</td>
<td>once (C)</td>
<td></td>
<td>125 F</td>
<td></td>
<td></td>
<td>Steiss et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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**Immuno/ Lymphoret**

<table>
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<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>21</td>
<td>Rat (Sprague-Dawley)</td>
<td>once (G)</td>
<td></td>
<td>150 M</td>
<td>300 M (vascular damage in the CNS)</td>
<td>Elo et al. 1988</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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**Neurological**

<table>
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<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>22</td>
<td>Rat (Fischer-344) (GO)</td>
<td>once (GO)</td>
<td></td>
<td>75</td>
<td>250 (altered gait and increased motor activity 1 day post-dosing)</td>
<td>Mattsson et al. 1997</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td></td>
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<tr>
<td>23</td>
<td>Rat (Wistar)</td>
<td>6 d (F)</td>
<td></td>
<td>15 F</td>
<td>(altered maternal behavior)</td>
<td>Sturtz et al. 2008</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<tr>
<td>24</td>
<td>Dog (Mongrel)</td>
<td>once (C)</td>
<td></td>
<td>125 F</td>
<td></td>
<td></td>
<td>Steiss et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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</table>

NOAELs are for histopathology of lymph nodes and spleen.
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Reproductive</td>
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<td></td>
</tr>
<tr>
<td>25</td>
<td>Mouse (ICR)</td>
<td>10 d Gd 0-9 (W)</td>
<td></td>
<td>100 F</td>
<td></td>
<td></td>
<td>Dinamarcia et al. 2007</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no changes in corpora lutea, implantations and resorption sites.</td>
</tr>
<tr>
<td>Developmental</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Charles et al. 2001</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no changes in litter data, fetal body weight and teratogenicity.</td>
</tr>
<tr>
<td>26</td>
<td>Rat (Fischer-344)</td>
<td>10 d Gd 6-15 1 x/d (GW)</td>
<td></td>
<td>75</td>
<td></td>
<td></td>
<td>Chernoff et al. 1990</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Rat (Sprague-Dawley)</td>
<td>10 d Gd 6-15 1 x/d (GW)</td>
<td></td>
<td>115 F</td>
<td>(increased incidence of supernumerary ribs)</td>
<td></td>
<td>Fofana et al. 2000</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Rat (Wistar)</td>
<td>9 d Gd 6-15 1 x/d (GO)</td>
<td></td>
<td>50</td>
<td></td>
<td>70</td>
<td>Fofana et al. 2002</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Rat (Wistar)</td>
<td>10 d Gd 6-15 1 x/d (GW)</td>
<td></td>
<td>70</td>
<td>(increased resorptions; renal malformations)</td>
<td></td>
<td>Fofana et al. 2002</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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</tr>
<tr>
<td>30</td>
<td>Rat (Sprague-Dawley)</td>
<td>Gd 6-15 10 d 1 x/d (GO)</td>
<td></td>
<td>25 F</td>
<td>50 F</td>
<td>(reduced fetal weight; increased incidence of soft-tissue and skeletal anomalies)</td>
<td>Schwetz et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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</tr>
</tbody>
</table>
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>Mouse (CD-1)</td>
<td>5 d Gd 8-12 1 x/d (GO)</td>
<td></td>
<td>87.5</td>
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<td>Kavlock et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>(reduced neonatal weight on postnatal day 1)</td>
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<tr>
<td>32</td>
<td>Hamster (Golden Syrian)</td>
<td>Gd 6-10 5 d 1x/d (GO)</td>
<td></td>
<td>100 F</td>
<td></td>
<td>Collins and Williams 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for teratogenicity.</td>
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<tr>
<td>33</td>
<td>Rabbit (New Zealand)</td>
<td>13 d Gd 6-18 1 x/d (GW)</td>
<td></td>
<td>90</td>
<td></td>
<td>Charles et al. 2001</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
</tbody>
</table>

**INTERMEDIATE EXPOSURE**

**Death**

| 34            | Dog (Mongrel)    | 13 wk 5 d/wk (C)                  |                   | 20                      |                     | Drill and Hiratzka 1953 | 2,4-dichlorophenoxyacetic acid | (3 out 4 dogs died on days 18, 25, and 49) |

**Systemic**

| 35            | Rat (Wistar)     | 28 d Gd 16-21 Pnd 1-23 (F)        | Bd Wt             | 70 M                    |                     | Bortolozzi et al. 1999 | 2,4-dichlorophenoxyacetic acid | (11% reduced body weight on Pnd 90) |

**OFFSPRING**

| 39            |                  |                                     |                   |                         |                     |                        |                           |                                    |
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/ Duration/ Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>Rat (Fischer- 344)</td>
<td>13 wk ad lib (F)</td>
<td>Resp</td>
<td>300</td>
<td></td>
<td></td>
<td>Charles et al. 1996a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Cardio</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastro</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hemato</td>
<td>15</td>
<td>100 (decreased platelets)</td>
<td></td>
<td></td>
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<tr>
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<td>Musc/skel</td>
<td>300</td>
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<tr>
<td></td>
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<td></td>
<td>Hepatic</td>
<td>100</td>
<td>300 (hepatocellular hypertrophy)</td>
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<td></td>
<td>Renal</td>
<td>15</td>
<td>100 (increased relative kidney weight)</td>
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<tr>
<td></td>
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<td></td>
<td>Endocr</td>
<td>15 F</td>
<td>100 F (decreased serum T3 and T4; adrenal cortex hypertrophy)</td>
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<td></td>
<td></td>
<td>Ocular</td>
<td>100 F</td>
<td></td>
<td>300 F (cataracts)</td>
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<td></td>
<td>Bd Wt</td>
<td>100</td>
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<td>300 (38-57% reduced weight gain)</td>
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<td>Metab</td>
<td>300</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
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<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>LOAEL</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tr>
<td>37</td>
<td>Rat (Fischer-344)</td>
<td>13 wk ad lib (F)</td>
<td>Resp</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Cardio</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td></td>
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<td>Gastro</td>
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<td>Hemato</td>
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<td></td>
<td></td>
<td>Renal</td>
<td>15 45 (degenerative changes in renal cortex)</td>
<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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</table>
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
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<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>38</td>
<td>Rat (Fischer-344) ad lib (F)</td>
<td>52 wk (ad lib (F))</td>
<td>Resp</td>
<td>45</td>
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<td></td>
<td>Cardio</td>
<td>45</td>
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EPA 1985
2,4-dichlorophenoxyacetic acid

EPA 1986
Hepatic NOAEL is for liver histopathology
2,4-dichlorophenoxyacetic acid
Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<td>60 F</td>
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<td>(decreased serum T4)</td>
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<td>Bd Wt</td>
<td>100 F</td>
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<td>Rat (CD)</td>
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<td>Marty et al. 2013</td>
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<td>Renal</td>
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<td>50 F</td>
<td>(decreased serum T3 and T4 and increased TSH on Gd 17)</td>
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<td>43</td>
<td>Rat (Fischer-344)</td>
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<td>75 F</td>
<td>150 F</td>
<td>(pale foci in the lungs)</td>
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<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAELs are for tissue histopathology.</td>
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<td></td>
<td>Ocular</td>
<td>75 F</td>
<td>150 F</td>
<td>(retinal degeneration)</td>
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<td>Bd Wt</td>
<td>75</td>
<td>150</td>
<td>(10% reduced terminal body weight)</td>
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<td>Rat (albino)</td>
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<td>100 F</td>
<td>(40-54% reduced maternal weight gain during pregnancy)</td>
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<td>2,4-dichlorophenoxyacetic acid</td>
<td>Mazhar et al. 2014</td>
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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>Key to Figure</th>
<th>Species (Strain)</th>
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<th>Less Serious (mg/kg/day)</th>
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<td>45</td>
<td>Rat (Sprague-Dawley)</td>
<td>3 mo ad lib (F)</td>
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<td>215 M</td>
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<td>Ozaki et al. 2001</td>
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<td>Renal</td>
<td>1.5 M</td>
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<td>Rat (Sprague-Dawley)</td>
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<td>6 M</td>
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<td>(reduced serum prolactin)</td>
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<td>2,4-dichlorophenoxyacetic acid</td>
<td>Milk ejection was reduced in all treated groups.</td>
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<td>Rat (Wistar)</td>
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<td>(increased liver weight and serum transaminases; liver histopathology)</td>
<td>Troudi et al. 2012a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

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<td>5 M 15 M (histological alterations in renal cortex)</td>
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**Reference**

EPA 1984

2,4-dichlorophenoxyacetic acid
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<td>178.9 M 429.4 M (lesions in renal tubule epithelial cells)</td>
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<td>Bd Wt</td>
<td>178.9 M 429.4 M (18% reduction in terminal body weight)</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<td>Hamster (Golden Syrian)</td>
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<td>2,4-dichlorophenoxyacetic acid</td>
<td>Charles et al. 1996c</td>
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<td>Cardio</td>
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<td>Gastro</td>
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<td>Musc/skel</td>
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<tr>
<td></td>
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<td>Hepatic</td>
<td>3.75</td>
<td>7.5</td>
<td>(perivascular active inflammation in the liver)</td>
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<td>Renal</td>
<td>1</td>
<td>3.75</td>
<td>(increased BUN and serum creatinine)</td>
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<td></td>
<td></td>
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<td>Bd Wt</td>
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<td>Metab</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

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<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>LOAEL</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
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<tr>
<td>55</td>
<td>Dog (Beagle)</td>
<td>1 yr ad lib (F)</td>
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<td></td>
<td></td>
<td>Charles et al. 1996c</td>
</tr>
<tr>
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<td>Cardio</td>
<td>7.5</td>
<td></td>
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<td>Gastro</td>
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<td>Hemato</td>
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<td>Musc/skel</td>
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</tr>
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<td></td>
<td>Hepatic</td>
<td>1</td>
<td>5</td>
<td>(increased serum cholesterol; perivascular inflammation of liver)</td>
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<td></td>
<td>Renal</td>
<td>1</td>
<td>5</td>
<td>(increased BUN and creatinine; tubular epithelium pigmentation)</td>
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<td></td>
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<td>Endocr</td>
<td>7.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Bd Wt</td>
<td>5 F</td>
<td></td>
<td>7.5 F (64% reduction in weight gain)</td>
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<td>Metab</td>
<td>1</td>
<td>5</td>
<td>(decreased serum glucose)</td>
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#### Immuno/ Lymphoret

<table>
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<tr>
<th>Key to Figure</th>
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<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>LOAEL</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>56</td>
<td>Rat (Fischer-344)</td>
<td>13 wk ad lib (F)</td>
<td></td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td>Charles et al. 1996a</td>
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</tbody>
</table>

*NOAELs are for tissue histopathology.*

*2,4-dichlorophenoxyacetic acid*
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>Rat (Fischer-344)</td>
<td>13 wk ad lib (F)</td>
<td></td>
<td>45</td>
<td></td>
<td></td>
<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of spleen and thymus.</td>
</tr>
<tr>
<td>58</td>
<td>Rat (Fischer-344)</td>
<td>52 wk ad lib (F)</td>
<td></td>
<td>45</td>
<td></td>
<td></td>
<td>EPA 1985</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of lymphoreticular tissues.</td>
</tr>
<tr>
<td>59</td>
<td>Rat (Fischer-344)</td>
<td>13 wk ad lib (F)</td>
<td></td>
<td>150</td>
<td></td>
<td></td>
<td>Gorzinski et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of lymphoreticular tissues.</td>
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<tr>
<td>60</td>
<td>Rat (CD)</td>
<td>30 d (F)</td>
<td></td>
<td>75.3 M</td>
<td></td>
<td></td>
<td>Marty et al. 2013</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no changes in humoral immune response and NK cell activity in F1 adult rats.</td>
</tr>
<tr>
<td>61</td>
<td>Rat (CD)</td>
<td>M: 11 wk F: 10 wk ad lib (F)</td>
<td></td>
<td>50 F</td>
<td></td>
<td></td>
<td>Marty et al. 2013</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no changes in weight and histology of lymphoreticular organs.</td>
</tr>
<tr>
<td>62</td>
<td>Mouse (B6C3F1)</td>
<td>13 wk ad lib (F)</td>
<td></td>
<td>90</td>
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<td></td>
<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of lymphoreticular tissues.</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
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<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
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<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tbody>
<tr>
<td>63</td>
<td>Mouse (B6C3F1)</td>
<td>52 wk ad lib (F)</td>
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<td>45</td>
<td></td>
<td></td>
<td></td>
<td>EPA 1987a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>64</td>
<td>Dog (Beagle)</td>
<td>13 weeks ad lib (F)</td>
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<td>7.5</td>
<td></td>
<td></td>
<td></td>
<td>Charles et al. 1996c</td>
<td>2,4-dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>65</td>
<td>Dog (Beagle)</td>
<td>1 yr ad lib (F)</td>
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<td>Charles et al. 1996c</td>
<td>2,4-dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>Neurological</td>
<td>66 Rat (Fischer- 344)</td>
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<td>Charles et al. 1996a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>67</td>
<td>Rat (Fischer- 344)</td>
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<td>45</td>
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<td></td>
<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>68</td>
<td>Rat (Fischer- 344)</td>
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<td>EPA 1985</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>Less Serious (mg/kg/day)</th>
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<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>69</td>
<td>Rat (Fischer-344)</td>
<td>13 wk ad lib (F)</td>
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<td>150</td>
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<td></td>
<td></td>
<td></td>
<td>Gorzinski et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of central and peripheral neural tissues.</td>
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<td>70</td>
<td>Rat (CD)</td>
<td>20 d (F)</td>
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<td>81.7 M</td>
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<td></td>
<td>Marty et al. 2013</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for neurobehavioral and neuropathological changes in adult F1 generation.</td>
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<td>71</td>
<td>Rat (CD)</td>
<td>M: 11 wk F: 10 wk ad lib (F)</td>
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<td>50 F</td>
<td></td>
<td></td>
<td>Marty et al. 2013</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for weight and histopathology of the brain.</td>
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<td>72</td>
<td>Rat (Fischer-344)</td>
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<td>75 150</td>
<td>(increased forelimb grip strength)</td>
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<td>Mattsson et al. 1997</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<tr>
<td>73</td>
<td>Rat (Fischer-344)</td>
<td>5 wk 2 d/wk (GO)</td>
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<td>20 M (increased forelimb grip strength)</td>
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<td>Squibb et al. 1983</td>
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<td>74</td>
<td>Rat (Fischer-344)</td>
<td>4 wk 7 d/wk (GO)</td>
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<td>Squibb et al. 1983</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>Exposure/Duration/Frequency (Route)</th>
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<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<td>75</td>
<td>Mouse (B6C3F1)</td>
<td>13 wk ad lib (F)</td>
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<td>90</td>
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<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of brain, spinal cord, and sciatic nerve.</td>
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<tr>
<td>76</td>
<td>Mouse (B6C3F1)</td>
<td>52 wk ad lib (F)</td>
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<td>45</td>
<td></td>
<td></td>
<td>EPA 1987a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of brain, spinal cord, and sciatic nerve.</td>
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<td>77</td>
<td>Dog (Beagle)</td>
<td>13 weeks ad lib (F)</td>
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<td>7.5</td>
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<td></td>
<td>Charles et al. 1996c</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of central and peripheral nervous system.</td>
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<td>78</td>
<td>Dog (Beagle)</td>
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<td>7.5</td>
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<td>Charles et al. 1996c</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of central and peripheral nervous system.</td>
</tr>
<tr>
<td>79</td>
<td>Rat (Fischer- 344)</td>
<td>13 wk ad lib (F)</td>
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<td>300</td>
<td></td>
<td></td>
<td>Charles et al. 1996a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs.</td>
</tr>
<tr>
<td>80</td>
<td>Rat (Fischer- 344)</td>
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<td>45</td>
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<td></td>
<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs.</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
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<th>NOAEL (mg/kg/day)</th>
<th>LOAEL</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<td>81</td>
<td>Rat (Fischer-344)</td>
<td>52 wk ad lib (F)</td>
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<td>45</td>
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<td></td>
<td>EPA 1985</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs.</td>
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<td>82</td>
<td>Rat (Fischer-344)</td>
<td>40 wk ad lib (F)</td>
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<td>80</td>
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<td></td>
<td></td>
<td>EPA 1986</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no changes in fertility and histopathology of ovaries and testes.</td>
</tr>
<tr>
<td>83</td>
<td>Rat (Fischer-344)</td>
<td>13 wk ad lib (F)</td>
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<td>150</td>
<td></td>
<td></td>
<td></td>
<td>Gorzinski et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs of males and females.</td>
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<td>84</td>
<td>Rat (Osborne-Mendel)</td>
<td>3-gen ad lib (F)</td>
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<td>Hansen et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no alterations in fertility.</td>
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<td>Rat (albino)</td>
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<td>Joshi et al. 2012</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td>86</td>
<td>Rat (CD)</td>
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<td>45.3 M</td>
<td>50 F</td>
<td></td>
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<td>Marty et al. 2013</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for no alterations in reproductive indices.</td>
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<tr>
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<td>Species (Strain)</td>
<td>Exposure/Duration/Frequency (Route)</td>
<td>System</td>
<td>NOAEL (mg/kg/day)</td>
<td>LOAEL (mg/kg/day)</td>
<td>Reference</td>
<td>Chemical Form</td>
<td>Comments</td>
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<td>Rat (Sprague-Dawley)</td>
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<td>Saghir et al. 2013</td>
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<td>NOAEL is for no alterations in reproductive indices.</td>
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<td>88</td>
<td>Mouse (B6C3F1)</td>
<td>13 wk ad lib (F)</td>
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<td>90</td>
<td></td>
<td>EPA 1984</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs.</td>
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<tr>
<td>89</td>
<td>Mouse (B6C3F1)</td>
<td>52 wk ad lib (F)</td>
<td></td>
<td>45</td>
<td></td>
<td>EPA 1987a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs.</td>
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</tr>
<tr>
<td>90</td>
<td>Dog (Beagle)</td>
<td>13 weeks ad lib (F)</td>
<td></td>
<td>7.5</td>
<td></td>
<td>Charles et al. 1996c</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs of males and females.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>Dog (Beagle)</td>
<td>1 yr ad lib (F)</td>
<td></td>
<td>7.5</td>
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<td>Charles et al. 1996c</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is histopathology of reproductive organs.</td>
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<tr>
<td>Developmental</td>
<td>Rat (Wistar)</td>
<td>28 d Gd 16-21 Pnd 1-23 (F)</td>
<td></td>
<td>70 (reduced preweaning pup's weight; neurobehavioral alterations in pups)</td>
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<td>Bortolozzi et al. 1999</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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## Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>NOAEL (mg/kg/day)</th>
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<td>93</td>
<td>Rat (Fischer- 344)</td>
<td>40 wk ad lib (F)</td>
<td>10</td>
<td>35 (14-16% reduced pup body weight on Pnd 28)</td>
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<td>EPA 1986</td>
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<td>94</td>
<td>Rat (Osborne-Mendel)</td>
<td>3-gen ad lib (F)</td>
<td>37</td>
<td></td>
<td>111 (reduced pup’s body weight and viability)</td>
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<td>Hansen et al. 1971</td>
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<td>95</td>
<td>Rat (CD)</td>
<td>M: 11 wk F: 10 wk ad lib (F)</td>
<td>9 F (reduced weight of pups on Pnd 22)</td>
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<td>Marty et al. 2013</td>
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<td>96</td>
<td>Rat (albino)</td>
<td>20 d GD 1-19 1 x/d (GO)</td>
<td>100 (31% reduced fetal weight; morphological and skeletal defects)</td>
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<td>Mazhar et al. 2014</td>
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<td>Rat (Sprague-Dawley)</td>
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<td>50 (decreased pup’s weight on Pnd 14)</td>
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<td>Saghir et al. 2013</td>
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<td>Rat (Wistar)</td>
<td>Ppd 1-16 (F)</td>
<td>2.5 (significant reduction in postnatal pup’s weight)</td>
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<td>Sturtz et al. 2010</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>LOAEL</th>
<th>Less Serious (mg/kg/day)</th>
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<th>Reference</th>
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<td>99</td>
<td>Rat (Wistar)</td>
<td>Gd 14-21 Pnd 0-14 ad lib (W)</td>
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<td>126</td>
<td>(17% reduction in pup's weight; liver histopathology)</td>
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<td>Troudi et al. 2012a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>Effects are on pups on Pnd 14.</td>
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<td>100</td>
<td>Rat (Wistar)</td>
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<td>126</td>
<td>(17% reduced pup's weight on Pnd 14; bone histopathology)</td>
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<td>Troudi et al. 2012b</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<td>NOAELs are for organ histopathology.</td>
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<td>150</td>
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<tr>
<td>Hemato</td>
<td>5 F</td>
<td>Hemato</td>
<td>5 F</td>
<td>75 F</td>
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<td></td>
<td></td>
<td></td>
<td>(decrease platelets, erythrocyte counts, and hematocrit)</td>
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<td>Musc/skel</td>
<td>150</td>
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<td>Hepatic</td>
<td>5 M</td>
<td>75 M</td>
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<td>(increased serum ALT activity)</td>
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<td>150</td>
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<td>(decrease serum T4)</td>
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<td></td>
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<td>(retinal degeneration, cataracts)</td>
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<td>Bd Wt</td>
<td>5 F</td>
<td>75 F</td>
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<td></td>
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<td>(11% reduced weight gain)</td>
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<td>Metab</td>
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<td>(43% reduced weight gain)</td>
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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
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<th>Key to Figure</th>
<th>Species (Strain)</th>
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<th>NOAEL (mg/kg/day)</th>
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<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tr>
<td>102</td>
<td>Rat (Osborne-Mendel)</td>
<td>2 yr ad lib (F)</td>
<td>Resp</td>
<td>92.5</td>
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<td>Hansen et al. 1971</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>Reference</th>
<th>Chemical Form</th>
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<tr>
<td>103</td>
<td>Mouse (B6C3F1)</td>
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<td>Resp</td>
<td>300 F</td>
<td>Charles et al. 1996b</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAELs are for organ histopathology.</td>
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<td>Gastro</td>
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<td>Musc/skel</td>
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<td>Renal</td>
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<td>62.5 M (degeneration/regeneration proximal tubule)</td>
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<td>150 F (degeneration/regeneration proximal tubule)</td>
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<td>Endocr</td>
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<td>Bd Wt</td>
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### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

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<th>NOAEL (mg/kg/day)</th>
<th>LOAEL Less Serious (mg/kg/day)</th>
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<td>15 M (increased absolute and relative adrenals weight)</td>
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<td>10</td>
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<td>Hansen et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
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<td>106 Rat (Fischer- 344)</td>
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<td>Hansen et al. 1971</td>
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<td>2 yr ad lib (F)</td>
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<td>10</td>
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<td></td>
<td>Hansen et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAELs are for histopathology of spleen and lymph nodes.</td>
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<td>Rat (Fischer-344)</td>
<td>2 yr ad lib (F)</td>
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<td>Charles et al. 1996b</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of central and peripheral neural tissues.</td>
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<td>Mouse (B6C3F1)</td>
<td>2 yr ad lib (F)</td>
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<td>300 F</td>
<td></td>
<td></td>
<td>Charles et al. 1996b</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for central and peripheral neural tissue histopathology.</td>
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<td>EPA 1987a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of central and peripheral nervous tissues.</td>
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<td>Dog (Beagle)</td>
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<td>Hansen et al. 1971</td>
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<td>NOAELs are for histopathology of brain and spinal cord.</td>
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<td>Charles et al. 1996b</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of reproductive organs of males and females.</td>
</tr>
</tbody>
</table>
### Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

<table>
<thead>
<tr>
<th>Key to Figure</th>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL (mg/kg/day)</th>
<th>Less Serious (mg/kg/day)</th>
<th>Serious (mg/kg/day)</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>Rat (Osborne-Mendel)</td>
<td>2 yr ad lib (F)</td>
<td></td>
<td>92.5</td>
<td></td>
<td></td>
<td>Hansen et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of the reproductive organs.</td>
</tr>
<tr>
<td>117</td>
<td>Mouse (B6C3F1)</td>
<td>2 yr ad lib (F)</td>
<td></td>
<td>125 M</td>
<td></td>
<td></td>
<td>Charles et al. 1996b</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of the reproductive organs.</td>
</tr>
<tr>
<td>118</td>
<td>Mouse (B6C3F1)</td>
<td>104 wk ad lib (F)</td>
<td></td>
<td>45</td>
<td></td>
<td></td>
<td>EPA 1987a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for histopathology of the reproductive organs.</td>
</tr>
<tr>
<td>119</td>
<td>Dog (Beagle)</td>
<td>2 yr ad lib (F)</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>Hansen et al. 1971</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAELs are for histopathology of the reproductive organs.</td>
</tr>
</tbody>
</table>

**a** The number corresponds to entries in Figure 3-2.

**b** Differences in levels of health effects between male and female are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

**c** Used to derive an intermediate-duration oral (MRL) of 0.009 mg/kg/day for 2,4-D. Using benchmark-dose modeling, a BMDRD05 of 1.27 mg 2,4-D/kg/day and a BMDLRD05 of 0.93 mg 2,4-D/kg/day, respectively, were calculated for reduced rat offspring body weight from the selected model (Exponential model 4). The BMDLRD05 was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive the MRL of 0.009 mg/kg/day. The intermediate-duration oral MRL was also adopted as acute-duration oral MRL for 2,4-D.

ad lib = ad libitum; ALT = alanine aminostransferase; Bd Wt = body weight; BUN = blood urea nitrogen; (C) = capsule; Cardio = cardiovascular; CNS = central nervous system; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; gen = generation; Gn pig = guinea pig; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolism; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Pnd = post-natal day; Ppd = post-parturition day; Resp = respiratory; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; x = time(s); (W) = drinking water; wk = week(s); yr = year(s)
Figure 3-2  Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (Continued)

Intermediate (15-364 days)

Systemic

mg/kg/day

Death  Respiratory  Cardiovascular  Gastrointestinal  Hematological

1000  100  10  1  0.1  0.01  0.001

Cancer Effect Level-Animals
LOAEL, More Serious-Animals
LOAEL, Less Serious-Animals
NOAEL - Animals

Cancer Effect Level-Humans
LOAEL, More Serious-Humans
LOAEL, Less Serious-Humans
NOAEL - Humans

LD50/LC50
Minimal Risk Level
for effects
other than Cancer

---

***DRAFT FOR PUBLIC COMMENT***
Figure 3-2  Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (Continued)
Intermediate (15-364 days)

Systemic

mg/kg/day

Musculoskeletal  Hepatic  Renal  Endocrine

Intermediate (15-364 days)

2,4-D

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Cancer Effect Level-Animals
LOAEL, More Serious-Animals
LOAEL, Less Serious-Animals
NOAEL - Animals
LD50/LC50

Cancer Effect Level-Humans
LOAEL, More Serious-Humans
LOAEL, Less Serious-Humans
NOAEL - Humans

Minimal Risk Level for effects other than Cancer

Animals

C-Cat  k-Monkey  f-Ferret  n-Mink  Cancer Effect Level-Animals  LOAEL, More Serious-Animals  NOAEL - Animals
D-Dog  m-Mouse  j-Pigeon  o-Other  LOAEL, Less Serious-Animals  NOAEL - Humans
R-Rat  h-Rabbit  e-Gerbil  s-Hamster  LOAEL, More Serious-Humans  NOAEL - Humans
P-Pig  a-Sheep  q-Cow  g-Guinea Pig  LD50/LC50
Figure 3-2  Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (Continued)
Intermediate (15-364 days)

Systemic
Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (Continued)
Intermediate (15-364 days)
Figure 3-2  Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (Continued)

Chronic (≥365 days)

mg/kg/day

Respiratory  Cardiovascular  Gastrointestinal  Hematological  Musculoskeletal  Hepatic  Renal  Endocrine  Dermal  Ocular  Body Weight  Metabolic  Immunologic  Lymphoid  Neurological  Reproductive

○ 103m ○103m ○103m ○103m ○103m ○103m ○103m ○103m ○103m ○103m ○103m ○108m ○112m ○117m

○ 101r ○101r ○101r ○101r ○101r ○101r ○101r ○101r ○101r ○101r ○101r ○116r ○117m

○ 102r ○102r ○102r ○102r ○102r ○102r ○102r ○102r ○102r ○102r ○102r ○107r ○116r

○ 104m ○104m ○104m ○104m ○104m ○104m ○104m ○104m ○104m ○109m ○113m ○118m

○ 105d ○105d ○105d ○105d ○105d ○105d ○105d ○105d ○110d ○114d ○119d

○ 101r ○101r ○103m ○101r ○101r ○101r

○ 104m ○104m

---

c-Cat  k-Monkey  f-Ferret  n-Mink  Cancer Effect Level-Animals  Cancer Effect Level-Humans  LD50/LC50

d-Dog  m-Mouse  j-Pigeon  o-Other  LOAEL, More Serious-Animals  LOAEL, More Serious-Humans  Minimal Risk Level

r-Rat  h-Rabbit  e-Gerbil  s-Hamster  LOAEL, Less Serious-Animals  LOAEL, Less Serious-Humans  for effects

p-Pig  a-Sheep  g-Guinea Pig  NOAEL - Animals  NOAEL - Humans  other than

q-Cow  Cancer

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

**Cardiovascular Effects.** 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 alterations were reported in the heart from dogs following administration of a single dose of ≤125 mg 2,4-D/kg (Steiss et al. 1987). In intermediate-duration studies, no effects were reported in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; 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 studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; 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.
Gastrointestinal Effects. Nausea and vomiting has 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.

For the most part, information regarding gastrointestinal effects in animals is limited to results of morphological examination of the gastrointestinal tract. 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. 1996a; 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 studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; 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.

Hematological Effects. The only information available in humans following oral exposure to 2,4-D is that apparent leukocytosis occurred in two of the four cases of intoxication with products containing 2,4-D described by Durakovic et al. (1992). No other relevant information was located.
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No information was located regarding hematological effects in animals in acute-duration studies. Intermediate- and chronic-duration 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. 1996a). 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. 1996a). 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).

A chronic-duration study reported that 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. 1996b). 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. 1996b), suggesting that mice are less susceptible than rats to 2,4-D-induced hematological effects.

Musculoskeletal Effects. 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).
Intermediate-duration studies provide information on skeletal muscle and bone morphology after oral exposure to 2,4-D. No significant effects were reported in rats exposed to \( \leq 300 \text{ mg kg}^{-1} \text{ day}^{-1} \) for intermediate durations (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1997), mice exposed to \( \leq 90 \text{ mg kg}^{-1} \text{ day}^{-1} \) (EPA 1984, 1987a), or dogs exposed to \( \leq 7.5 \text{ mg kg}^{-1} \text{ day}^{-1} \) (Charles et al. 1996c).

Similar results were reported in chronic-duration studies in rats exposed to \( \leq 150 \text{ mg kg}^{-1} \text{ day}^{-1} \) (Charles et al. 1996b; Hansen et al. 1971), mice exposed to \( \leq 300 \text{ mg kg}^{-1} \text{ day}^{-1} \) (Charles et al. 1996b; EPA 1987a), and dogs exposed to \( \leq 10 \text{ mg kg}^{-1} \text{ day}^{-1} \) (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.

**Hepatic Effects.** 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.

Limited data from acute-duration 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).

More information is available regarding hepatic effects in animals in longer-term studies, especially intermediate-duration studies. Results in rats show apparent inconsistencies between studies. In general, results 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...
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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, doses of ≥150 mg 2,4-D/kg/day induced histological alterations in the liver and the NOAEL was 100 mg/kg/day (Charles et al. 1996a; EPA 1984; Gorzinski et al. 1987). A 2-generation reproductive study reported a NOAEL of 80 mg 2,4-D/kg/day for liver histopathology in the parental and F1 generations (EPA 1986).

In mice, exposures 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, 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).

Chronic-duration 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 150 mg 2,4-D/kg/day in the 13-week study (Gorzinski et al. 1987). Rats exposed for 2 years to 150 mg 2,4-D/kg/day showed only increased incidence of “minimal panlobular tinctorial properties,” exposure to 75 mg/kg/day increased serum ALT activity, and the NOAEL was 5 mg/kg/day (Charles et al. 1996b). In mice, exposure for 2 years to ≤300 mg 2,4-D/kg/day did not induce liver histopathology (Charles et al. 1996b) and the same was reported in dogs exposed for 2 years up to 10 mg 2,4-D/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).

Renal Effects. 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 that 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.

***DRAFT FOR PUBLIC COMMENT***
Only two acute-duration oral studies in dogs examined renal end points. No significant histopathological alterations were reported in the kidneys from dogs administered a single dose of 125 mg 2,4-D/kg (highest dose tested) (Steiss et al. 1987). A single dose of 200 mg 2,4-D/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 (Dickow et al. 2000).

Alterations in the kidneys have been reported in intermediate-duration oral studies in rats, but there are some apparent inconsistencies between studies. The lowest LOAEL was approximately 7.1 mg 2,4-D/kg/day reported in a 13-week study; the NOAEL was 1.5 mg/kg/day (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. This was not observed in hamsters exposed to ≤474 mg 2,4-D/kg/day for 13 weeks (Ozaki et al. 2001). Other 13-week or shorter duration studies in rats reported LOAELs for histopathological alterations in the kidneys at doses in the range of 40–60 mg 2,4-D/kg/day (EPA 1984; Gorzinski et al. 1987; Marty et al. 2013; Saghir et al. 2013). NOAELs ranged from 6 to 15 mg 2,4-D/kg/day. Renal clearance of 2,4-D is saturated at different levels in adult female (14–27 mg/kg/day) and adult male (approximately 63 mg/kg/day) rats (Saghir et al. 2013). However, Charles et al. (1996a) reported kidney histopathology in male and female rats only at 300 mg 2,4-D/kg/day for 13 weeks, but not after exposure to 100 mg/kg/day, and a 2-generation study reported a LOAEL of 20 mg/kg for kidney histopathology in rats (EPA 1987b). A 52-week study reported increased tubular cell brown pigments in male and female rats exposed to 15 mg 2,4-D/kg/day; females also showed fine vacuolization of the cytoplasm in the renal cortex at 15 mg/kg/day; the NOAEL was 5 mg/kg/day (EPA 1985). Chronic-duration studies did not report kidney lesions in rats exposed to ≤150 mg 2,4-D/kg/day for 2 years (Charles et al. 1996b; Hansen et al. 1971).

The picture is not clear in mice either. 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 exposed to 15 mg 2,4-D/kg/day for 13 or 52 weeks; NOAELs were 1–5 mg/kg/day (EPA 1984, 1987a). However, in another 13-week study, kidney lesions were reported in male mice after exposure to approximately 430 mg 2,4-D/kg/day, but not in mice exposed to approximately 179 mg/kg/day (Ozaki et al. 2001). Two-year exposures of mice to ≥15 mg 2,4-D/kg/day also resulted in kidney alterations; NOAELs were in the 1–5 mg/kg/day range (Charles et al. 1996b; EPA 1987a).
No histological alterations were seen in the kidneys from dogs exposed to ≤7.5 mg 2,4-D for intermediate durations, but there was some indication of altered kidney function assessed as increased BUN and serum creatinine (Charles et al. 1996c). Hansen et al. (1971) did not find morphological alterations in the kidneys from dogs exposed to ≤10 mg 2,4-D/kg/day for 2 years; however, clinical chemistry tests were not conducted in this study, so kidney function was not addressed.

Kidney effects were observed in all of the animal species tested, but with the wide range of results available, it is difficult to make generalizations.

**Endocrine Effects.** The only relevant information regarding endocrine effects in humans following oral exposure to 2,4-D is that acute congestion was seen in the adrenals in the lethal case reported by Nielsen et al. (1965) and that the endocrine system appeared normal at autopsy in the case reported by Dudley and Thapar (1972).

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.

Serum levels of prolactin were significantly decreased in rats administered doses ≥2.5 mg 2,4-D/kg/day on postpartum days 1–16 (Stürtz et al. 2008, 2010). This effect was attributed in part to decreased levels of serotonin and increased levels of dopamine in the arcuate nucleus of the brain (Stürtz et al. 2008, 2010).

Alterations in thyroid hormone levels have been reported in rats in long-term studies. For example, serum thyroxine (T4) and triiodothyronine (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. 1996a). 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; 1996a) 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 for 2 years; the NOAEL was 5 mg/kg/day (Charles et al. 1996b). 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. 1996a). 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.

In summary, the fact that relatively low doses of 2,4-D reduced serum levels of prolactin in postpartum rats is significant in that it resulted in reduced offspring body weight. In humans, prolactin is critical for the establishment of lactation, for milk production, and for an adequate milk macronutrient content (Ostrom 1990). 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. Further generational and neurological studies would be beneficial to add weight of the evidence to findings.

**Dermal Effects.** No information was located regarding dermal effects in humans following oral exposure to 2,4-D.

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).

**Ocular Effects.** No information was located regarding ocular effects in humans following oral exposure to 2,4-D.
Ocular effects were reported in rats in intermediate- and chronic-duration 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 intermediate-duration 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. 1996a).

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. 1996b). 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.

**Body Weight Effects.** No information was located regarding body weight effects in humans following oral exposure to 2,4-D.

Many 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
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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 studies in rats provide a less-than-clear picture. Three studies reported a NOAEL of 100 mg 2,4-D/kg/day (Charles et al. 1996a; Gorzinski et al. 1987; Saghir et al. 2013). Doses ≥150 mg 2,4-D/kg/day significantly decreased body weight gain (Charles et al. 1996a; 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 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).

Chronic-duration 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. 1996b) and a NOAEL of 300 mg/kg/day for mice (Charles et al. 1996b).

**Metabolic Effects.** Elevated potassium levels were reported prior to death in the case of an individual who may have ingested 25–35 g of 2,4-D from a commercial herbicide product (Keller et al. 1994). Metabolic acidosis was reported in three out of the four nonlethal cases of intoxication with preparations containing 2,4-D reported by Durakovic et al. (1992). No further human information was located.
Limited relevant data are available from studies in animals. Significantly reduced serum calcium and potassium were reported in dogs following administration of a single dose of 200 mg 2,4-D/kg in a gelatin capsule (Dickow et al. 2000). The investigators noted that these effects may have been secondary to vomiting and diarrhea also experienced by the dogs.

No significant alterations in electrolytes or glucose levels were reported in rats dosed with ≤300 mg 2,4-D/kg/day for 13 weeks (Charles et al. 1996a) or ≤150 mg/kg/day for 2 years (Charles et al. 1996b). Also, no significant metabolic alterations were reported in dogs exposed up to ≤7.5 mg 2,4-D/kg/day for 13 weeks, but exposure to ≥5 mg 2,4-D/kg/day for 52 weeks significantly reduced blood glucose (27–31%) in dogs (Charles et al. 1996c).

Based on limited data, it does not appear that metabolic alterations need to be a concern for humans exposed to environmentally levels of 2,4-D.

### 3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans following oral exposure to 2,4-D.

For the most part, 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 studies did not report morphological alterations in lymphoreticular tissues from rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; 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 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).
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Chronic-duration exposure of rats to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; 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.

NOAEL and LOAEL values for immune system effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

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 of the latter 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 dose levels tested, but some studies reported neurobehavioral and neurochemical alterations.
An acute-duration 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 dose of up to 125 mg 2,4-D/kg in a capsule (Steiss et al. 1987).

Intermediate-duration 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. 1996a). Other studies that examined this end point 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 in rats administered ≤150 mg 2,4-D/kg/day (Charles et al. 1996b), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; 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. Much 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).

In intermediate-duration 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
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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). 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.

NOAEL and LOAEL values for neurological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

Virtually no information was located regarding reproductive effects in humans following oral exposure to 2,4-D. 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).

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

Only one acute-duration 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 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. 1996a;
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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. 1996a). 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 studies (EPA 1986; Hansen et al. 1971; Marty et al. 2013; Saghir et al. 2013), and neither were mating index, time to mating, gestation length, pre- and postimplantation losses, and 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.

Additional intermediate-duration 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. 1996b; Hansen et al. 1971), mice exposed up to 300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), or dogs exposed 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.

NOAEL and LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.
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3.2.2.6 Developmental Effects

No information was located regarding developmental effects in humans following oral exposure to 2,4-D.

Developmental effects have been observed in rodents following perinatal exposure to 2,4-D. For the most part, results from acute-duration 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 yet similar studies in rats, 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).

Exposure of 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 was 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 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 studies provide information on developmental end points; all of the available studies were conducted in rats. The lowest LOAEL for developmental effects 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.

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to the litter by an action of 2,4-D at the central level. 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. With the changes in milk content, it is possible that nutritional deficiency occurred that resulted in hindered growth of the pups. Other studies have also reported effects on pup body weight, but at 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, reduced pup body weight (about 10%) was reported following perinatal exposure to approximately 9 mg 2,4-D/kg/day on PND 22 (Marty et al. 2013). The study by Stürtz et al. (2010) was used to derive an intermediate-duration oral MRL for 2,4-D. 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.

Other studies that reported reduced offspring weight at higher maternal 2,4-D 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 can induce reductions in offspring weight that are not always associated with maternal effects. It has caused alterations in
neurobehavioral effects in one study (Bortolozzi et al. 1999) and inhibited milk ejection at low maternal exposure levels in another study (Stürtz et al. 2010).

NOAEL and LOAEL values for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

### 3.2.2.7 Cancer

No information was located regarding cancer in humans following oral exposure to 2,4-D.

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. 1996b; Hansen et al. 1971), mice were similarly exposed to up to 300 mg 2,4-D/kg/day (Charles et al. 1996b; 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). 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 2016; Loomis et al. 2015). IARC has not yet released a full report in support for its recent classification.

### 3.2.3 Dermal Exposure

As mentioned in the introduction to Section 3.2.1, most of the information available regarding exposure to 2,4-D and health end points in humans comes from studies of individual 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. Therefore, studies of humans involved in these activities are summarized in this section. 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.

### 3.2.3.1 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 to 3 mg/m³ 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 3.2.3.7, Cancer).
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The only information available from studies in animals is that the dermal LD$_{50}$ in rabbits was determined to be >2,000 mg/kg (Gorzinski et al. 1987).

3.2.3.2 Systemic Effects

No information was located regarding cardiovascular, musculoskeletal, or ocular effects in humans following dermal exposure to 2,4-D. No information was located regarding respiratory, gastrointestinal, cardiovascular, musculoskeletal, endocrine, or metabolic effects in animals following dermal exposure to 2,4-D. The highest NOAEL values and all LOAEL values from each reliable study for other systemic effects in each species and duration category are recorded in Table 3-3.

**Respiratory Effects.** In the Agricultural Health Study (AHS), use of 2,4-D was not associated with wheezing (odds ratio [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). 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 (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. In a group of 583 farm women 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).
### Table 3-3 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Dermal

<table>
<thead>
<tr>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>NOAEL</th>
<th>Less Serious</th>
<th>Serious</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
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<tr>
<td><strong>Death</strong></td>
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<td>Rabbit (New Zealand)</td>
<td>24 hr (GO)</td>
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<td>Gorzinski et al. 1987</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>The LD50 was greater than 2000 mg/kg.</td>
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<td><strong>Systemic</strong></td>
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<td></td>
<td></td>
<td>Kimura et al. 1998</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td>Dog hairless</td>
<td>7 d 1 x/d Dermal</td>
<td></td>
<td>0.036 mg</td>
<td>(slight epidermal thickening and hyperplasia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit (New Zealand)</td>
<td>4 hr Dermal</td>
<td></td>
<td>500 B mg</td>
<td></td>
<td></td>
<td>EPA 1992</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>NOAEL is for skin irritation.</td>
</tr>
<tr>
<td>Immuno/ Lymphoret</td>
<td>Mouse (BALB/c) 9 d</td>
<td></td>
<td>5 F Percent (%)</td>
<td>(respiratory allergen)</td>
<td></td>
<td>Fukuyama et al. 2009</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3-3 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Dermal (continued)

<table>
<thead>
<tr>
<th>Species (Strain)</th>
<th>Exposure/Duration/Frequency (Route)</th>
<th>System</th>
<th>LOAEL</th>
<th>Reference</th>
<th>Chemical Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>21 d 7 d/wk 6 h/d</td>
<td>Hemato</td>
<td>1000 B mg/kg/day</td>
<td>EPA 1991a</td>
<td>2,4-dichlorophenoxyacetic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatic</td>
<td>1000 B mg/kg/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal</td>
<td>100 F mg/kg/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000 F mg/kg/day</td>
<td></td>
<td></td>
<td>(increased absolute and relative kidney weight)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dermal</td>
<td>10 F mg/kg/day</td>
<td></td>
<td></td>
<td>(very slight erythema)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ocular</td>
<td>1000 B mg/kg/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bd Wt</td>
<td>1000 B mg/kg/day</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B = both sexes; Bd Wt = body weight; d = day(s); F = Female; (GO) = gavage in oil; Hemato = hematological; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; x = time(s); wk = week(s)
Gastrointestinal Effects. 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.

Hematological Effects. 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).

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). No further information was located.

Hepatic Effects. 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.

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.
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The only relevant information in animals 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 treatment-related alterations in clinical chemistry tests or histological alterations in the liver (EPA 1991a).

Renal Effects. In the cross-sectional study mentioned above (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.

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).

Application of 10–1,000 mg 2,4-D/kg/day onto the skin of male and female rabbits for 21 days significantly increased absolute and relative kidney weight in high-doses females (EPA 1991a). However, while kidney function tests and histology were performed, there were no treatment-related alterations in clinical chemistry for kidney function nor histological changes in the kidneys.

Endocrine Effects. 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≤0.05), insulin (p≤0.01), and TSH (p≤0.05), especially in populations with high serum glucose and low T4 levels.

Additional information regarding endocrine effects is available from the AHS. 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% 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 a positive association between ever-use of 2,4-D and hypothyroid disease (OR 1.35;
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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).

**Dermal Effects.** The only relevant information available 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).

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).

**Ocular Effects.** The only information regarding ocular effects in humans following exposure 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.

The only relevant information in animals 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).

**Body Weight Effects.** 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.
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).

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

**Metabolic Effects.** In a cross-sectional study of a subset of NHANES 1988–1994 subjects, serum levels of glucose and glycosylated hemoglobin (marker for mean plasma concentration of glucose over a prolonged period of time) in a group of 102 subjects with detectable levels of 2,4-D in the urine were not different from mean levels recorded in 625 subjects with urinary 2,4-D below the limit of detection (1 mg/dL) (Schreinemachers 2010).

### 3.2.3.3 Immunological and Lymphoreticular Effects

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.
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). No additional studies were located regarding immunological effects of 2,4-D in animals.

3.2.3.4 Neurological Effects

Information regarding neurological effects in humans following exposure 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 between 2,4-D and the disease. 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. 2006). 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 0.69–6.23) 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 parkinsonim 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).

It should also be mentioned that 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 [RR 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.

No studies were located regarding neurological effects in animals following dermal exposure to 2,4-D acid or simple salts.

### 3.2.3.5 Reproductive Effects

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 ($\geq0.1 \mu g/g$ creatinine) and semen quality (Swan et al. 2003).

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, that 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 studies were located regarding reproductive effects in animals following dermal exposure to 2,4-D.

### 3.2.3.6 Developmental Effects

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)
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(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.

No studies were located regarding developmental effects in animals following dermal exposure to 2,4-D.

3.2.3.7 Cancer

Cancers Affecting the Lymphatic System. Many studies, mostly population-based, case-control design, have examined the relationship 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).

Some studies have not found statistically significant 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 (RR 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-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-DP and 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 [RR] 0.97; 95% CI 0.77–1.22) (Goodman et al. 2015).

**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 standardized incidence ratio (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 72 colon cancer cases diagnosed in Kansas during 1976–1982 and 948 controls selected from the general population found an increased risk for farmers exposed to phenoxy herbicides than to other chemical groups (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, RRs for 2,4-D calculated using Poisson regression and controlling for confounding factors were not elevated (Engel et al. 2005). The RR 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) (Yin 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; 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.

Data regarding cancer in animals are limited to a case-control study of malignant lymphoma in household dogs from 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

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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 EPA has assigned 2,4-D to carcinogenicity Group D, “not classifiable as to human carcinogenicity” (EPA 2005a). 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 animals and limited evidence in experimental animals (IARC 2016; Loomis et al. 2015).

### 3.3 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 3-4 and 3-5 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.

**In vivo Exposure Studies.** Results from human *in vivo* exposure genotoxicity studies are mixed (Table 3-4). 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
### Table 3-4. Genotoxicity of 2,4-D In Vivo

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

#### Table 3-4. Genotoxicity of 2,4-D In Vivo

<table>
<thead>
<tr>
<th>Species (test system)</th>
<th>End point</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Drosophila melanogaster</em></td>
<td>Somatic mutation and recombination (wing spot test)</td>
<td>(+)</td>
<td>Kaya et al. 1999</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Somatic mutation (wing spot test)</td>
<td>+</td>
<td>Tripathy et al. 1993</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Sex-linked recessive mutation</td>
<td>+</td>
<td>Tripathy et al. 1993</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Sex-linked recessive mutation</td>
<td>(+)</td>
<td>Magnusson et al. 1977</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Sex-linked recessive mutation</td>
<td>+</td>
<td>Rasmuson and Svahlin 1978</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Sex-linked recessive mutation</td>
<td>(+)</td>
<td>Vogel and Chandler 1974</td>
</tr>
</tbody>
</table>

*Study conducted using 2,4-D sodium salt.

− = negative result; + = positive result; (+) = weak positive result; 2,4-D = 2,4-dichlorophenoxyacetic acid
### Table 3-5. Genotoxicity of 2,4-D *In Vitro*

<table>
<thead>
<tr>
<th>Species (test system)</th>
<th>End point</th>
<th>With activation</th>
<th>Without activation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prokaryotic organisms:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA1535, TA1535, TA1537, TA1538 (Ames test)</td>
<td>Gene mutation</td>
<td>–</td>
<td>–</td>
<td>Charles et al. 1999a</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA98, TA100</td>
<td>Gene mutation</td>
<td>–</td>
<td>–</td>
<td>Kubo et al. 2002</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA97, TA98, TA100, TA102; <em>Escherichia coli</em></td>
<td>Gene mutation/SOS chromatid test</td>
<td>–</td>
<td>–</td>
<td>Mersch-Sundermann et al. 1994</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>Mutation (host mediated assay)</td>
<td>No data</td>
<td>–</td>
<td>Styles 1973</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA1530, TA1535, TA1531, TA1583</td>
<td>Mutation</td>
<td>No data</td>
<td>–</td>
<td>Zetterberg et al. 1977a</td>
</tr>
<tr>
<td><em>S. typhimurium. uvrB, rec; E. coli; Bacillus subtilis rec</em></td>
<td>DNA damage</td>
<td>No data</td>
<td>+</td>
<td>Garrett et al. 1986</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Mutation (modified SOS microplate assay)</td>
<td>No data</td>
<td>–</td>
<td>Venkat et al. 1995</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> strain <em>D7ts1</em></td>
<td>Mitotic gene conversion; reverse mutation</td>
<td>No data</td>
<td>+</td>
<td>Venkov et al. 2000</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> strains D4, D5</td>
<td>Mitotic gene conversion; recombination</td>
<td>No data</td>
<td>+</td>
<td>Zetterberg et al. 1977a</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> strain RAD 18</td>
<td>Mitotic gene conversion; recombination</td>
<td>No data</td>
<td>+</td>
<td>Zetterberg 1978</td>
</tr>
<tr>
<td><strong>Eukaryotic organisms:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>Mutation (colony forming ability, single strand breaks)</td>
<td>No data</td>
<td>–</td>
<td>Clausen et al. 1990</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>Mutation (colony forming ability, single strand breaks)</td>
<td>No data</td>
<td>+</td>
<td>Clausen et al. 1990a</td>
</tr>
<tr>
<td>Human lymphocytes</td>
<td>Sister chromatid exchange</td>
<td>No data</td>
<td>+</td>
<td>Korte and Jalal 1982</td>
</tr>
<tr>
<td>Human lymphocytes (whole blood and leukocyte cultures)</td>
<td>Sister chromatid exchange</td>
<td>No data</td>
<td>+</td>
<td>Soloneski et al. 2007</td>
</tr>
<tr>
<td>Human lymphocytes</td>
<td>Sister chromatid exchange</td>
<td>No data</td>
<td>+</td>
<td>Turkula and Jalal 1985</td>
</tr>
<tr>
<td>Human lymphocytes</td>
<td>Chromosome aberrations</td>
<td>–</td>
<td>–</td>
<td>Mustonen et al. 1986</td>
</tr>
<tr>
<td>Human lymphoma and leukemia cells</td>
<td>Chromosome aberrations</td>
<td>No data</td>
<td>+</td>
<td>Venkov et al. 2000</td>
</tr>
</tbody>
</table>

***DRAFT FOR PUBLIC COMMENT***
### Table 3-5. Genotoxicity of 2,4-D In Vitro

<table>
<thead>
<tr>
<th>Species (test system)</th>
<th>End point</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human lymphocytes</td>
<td>Chromosome aberrations; micronucleus assay</td>
<td>+</td>
<td>Zeljegic and Garaj-Vrhovac 2004</td>
</tr>
<tr>
<td>Human lymphocytes</td>
<td>DNA damage</td>
<td>No data</td>
<td>Sandal and Yilmaz 2011</td>
</tr>
<tr>
<td>Chinese hamster (V79 cell culture)</td>
<td>Mutation</td>
<td>No data</td>
<td>Ahmed et al. 1977</td>
</tr>
<tr>
<td>Chinese hamster (CHO cells)</td>
<td>Chromosome aberrations</td>
<td>+</td>
<td>Galloway et al. 1987</td>
</tr>
<tr>
<td>Chinese hamster (CHO cells)</td>
<td>Sister chromatid exchange</td>
<td>–</td>
<td>Galloway et al. 1987</td>
</tr>
<tr>
<td>Chinese hamster (CHO cells)</td>
<td>Sister chromatid exchange</td>
<td>No data</td>
<td>González et al. 2005</td>
</tr>
<tr>
<td>Chinese hamster (CHO cells)</td>
<td>Sister chromatid exchange</td>
<td>–</td>
<td>Linnainmaa 1984</td>
</tr>
<tr>
<td>Chinese hamster (CHO cells)</td>
<td>DNA damage</td>
<td>No data</td>
<td>González et al. 2005</td>
</tr>
<tr>
<td>Rat (primary hepatocytes)</td>
<td>Unscheduled DNA synthesis</td>
<td>No data</td>
<td>Charles et al. 1999a</td>
</tr>
<tr>
<td>Syrian Golden Hamster embryo (SHE cells)</td>
<td>Morphological cell transformation, DNA damage</td>
<td>No data</td>
<td>Maire et al. 2007</td>
</tr>
<tr>
<td>Syrian Golden Hamster embryo (SHE cells)</td>
<td>Morphological cell transformation</td>
<td>No data</td>
<td>Mikalsen et al. 1990</td>
</tr>
</tbody>
</table>

*Study conducted using 2,4-D-sodium salt.

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
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 was 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. 1997; Rasmuson and Svahlin 1978; Volgel 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 3-5, 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 et al. 1977, 1978).

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 Jalal 1982; Sandal and Yılmaz 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 mainly positive results for mutation, chromosomal aberrations, sister chromatid exchange (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 in other studies 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, although results of genotoxicity studies in humans, animals, and *in vitro* studies have been mixed, the fact that some studies have reported positive results supports a biological plausibility of effects occurring as a result of exposure to 2,4-D and cannot be discounted.
3.4 TOXICOKINETICS

2,4-D is rapidly and almost completely absorbed from the gastrointestinal tract in humans and animals, but dermal absorption is relatively low (<10% of an applied dose in humans). 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. Studies in humans have estimated elimination half-lives in urine of <2 days following single oral or dermal doses of 2,4-D. In animals, 2,4-D can be transferred to fetal tissues and to offspring through maternal milk, although this has not been definitively proven in humans. The toxicokinetics of 2,4-D is species- and sex-dependent largely due to differences in renal clearance of 2,4-D. This differential capacity for excreting 2,4-D plays an important role in the susceptibility to 2,4-D-induced effects between species.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

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

3.4.1.2 Oral 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). Studies in animals have also shown sex differences as well as species differences in disposition of orally absorbed 2,4-D. For example, analysis of plasma concentrations of 2,4-D in rats following oral administration of a 5 mg/kg dose showed no difference in absorption rates between males and females. In a study in dogs and rats administered a single oral dose of 5 or 50 mg/kg 14C-2,4-D, rats eliminated radioactivity from plasma significantly faster than dogs (van Ravenzwaay et al. 2003). Approximate elimination half-lives were 1.3–3.4 hours in rats and 99–134 hours in dogs following the low- and high-dose, respectively. This resulted in areas under the curve (AUC\(_{0-\infty}\)) significantly higher in dogs than in the rats. In addition, over the monitoring period of 120 hours, elimination of radioactivity from plasma was complete in rats, but not in dogs.

3.4.1.3 Dermal Exposure

Dermal absorption of 2,4-D in humans is low compared to oral absorption. Male volunteers that received a topical application of 4 µg/cm² 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 that reported that 4.5% of an applied dose of 10 mg 2,4-D in acetone/water over a 9 cm² 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 over predict 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
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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-cm² area of abdominal skin lightly clipped resulted in absorption of 9.8% of the dose when the soil load was 1 mg/cm² and 15.9% when the soil load was 40 mg/cm². 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 also on the type of soil (Duff and Kissel 1996).

In a comparative study in which rats and guinea pigs were applied ¹⁴C-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 ¹⁴C-2,4-D in acetone penetrated the body (disappeared from the covered site of application) in 1 hour and about 21% in 24 hours (Grisson 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).

### 3.4.1.4 Other Routes of Exposure

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.

### 3.4.2 Distribution

#### 3.4.2.1 Inhalation Exposure

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

#### 3.4.2.2 Oral Exposure

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 14C-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. (2013) 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.
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3.4.2.3 Dermal Exposure

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.

3.4.2.4 Other Routes of Exposure

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 LD50) 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 14C-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 14C-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 14C-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 postnatal day (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.

3.4.3 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 3-3 shows a proposed metabolic pathway for 2,4-D in dogs.

### 3.4.4 Elimination and Excretion

#### 3.4.4.1 Inhalation Exposure

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 3.8.1 Biomarkers Used to Identify or Quantify Exposure to 2,4-D).

#### 3.4.4.2 Oral 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. 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
Figure 3-3. Proposed Metabolic Pathway of 2,4-D in Dogs

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

Source: Van Ravenzwaay et al. 2003
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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 14C-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 14C-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 3-3). 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.

3.4.4.3 Dermal Exposure

In volunteers applied a dermal dose of 4 µg/cm² 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 cm² 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.

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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/cm² 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 ¹⁴C-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 ¹⁴C-2,4-D in acetone on the shaved back excreted small amounts of radiolabel in the feces and as CO₂, 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.

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

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.
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The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. However, if the uptake and disposition of the chemical substance(s) are adequately described, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for 2,4-D exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

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
Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance

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

Source: Krishnan and Andersen 1994
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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 establish 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 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 (Smith et al. 1980).

3.4.5.1 Discussion of Models

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 processes: (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; Smith et al. 1980). 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) and 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 ($V_{\text{max}}, K_m$); (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; Smith et al. 1980, unpublished). 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 incudes 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 (Smith et al. 1980, unpublished). 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 (Ylatalo 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 (Smith et al. 1980).

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 (1977) 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/cm²). 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, $V_{\text{max}}$ 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. 1984, 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 (PHED Task Force 1995). 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 (Serota et al. 1983). 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.

### 3.5 MECHANISMS OF ACTION

#### 3.5.1 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. 1977a). 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 Ravenszwaay et al. 2003). The respectively values for dogs were 95.7 and 87.6%.
Metabolism. As indicated in Section 3.4.3, 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 Ravenszwaay 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 life-stage. Studies have shown that in rats, saturation occurs following single oral doses in excess of 50 mg/kg 2,4-D (Gorzinski et al. 1987; Saghir et al. 2013). Adult male rats express higher levels of OAT1 than adult females (Buist et al. 2002), which is consistent with an increased susceptibility of female rats to 2,4-D-induced renal lesions than male rats (Marty et al. 2013). The latter investigators suggested that saturation of the OAT1 at lower 2,4-D plasma concentrations than in males would preferentially decrease the delivered dose of 2,4-D to the proximal tubules in females relative to males. The differential expression of OAT1 in males 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 3.4.2.2 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 RNA in males than in females by PND 40 (Buist et al. 2002) could explain the findings of Saghir et al. (2013) 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.

### 3.5.2 Mechanisms of Toxicity

The role of oxidative stress in the toxicity of 2,4-D has been explored in a few studies. Twenty-five-day-old offspring from rats exposed to 100 mg 2,4-D/kg/day from PND 9 to 25 showed significantly 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).

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 summary below is taken 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.

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 D2 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 \textit{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 \textit{in vitro} possibly leading to decreased neurite outgrowth (Rosso et al. 2000b).
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3.5.3 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.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. However, appropriate terminology to describe such effects remains controversial. The terminology endocrine disruptors, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for “...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...”. To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning endocrine disruptors. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as hormonally active agents. The terminology endocrine modulators has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavonoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering,
for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought
to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994;

There are no studies in humans that would suggest that 2,4-D is an endocrine disruptor chemical. Studies
in animals or in vitro assays suggest that 2,4-D is not an endocrine disruptor. Although altered behavioral
effects may occur (Bortolozzi et al. 2001; Duffard et al. 1996; Rosso et al. 2000a, 2000b) that indicate a
disruption, it is unlikely that they occurred through endocrine pathways.

Studies in animals summarized in Section 3.2.2, Oral Exposure, did not find morphological alterations in
endocrine glands following exposure to 2,4-D. One study reported a significant reduction in serum
prolactin levels in rats dosed with ≥15 mg 2,4-D/kg/day via the drinking water on postpartum days 1–7
(Stürtz et al. 2008). The investigators suggested that alterations in levels of serotonin and dopamine in
the arcuate nucleus of the brain may have been responsible for the reduction in serum prolactin. Some
studies reported decreased serum levels of T4 in rats exposed to relatively high doses of 2,4-D (i.e.,
Charles et al. 1996a; Gorzinski et al. 1987), which could have been due to competition of 2,4-D with T4
for binding with plasma proteins. None of these studies reported histopathological changes in the thyroid
gland. In an F1-extended 1-generation reproductive study in rats, there was no evidence that 2,4-D had
androgenic, anti-androgenic, estrogenic, or anti-estrogenic activity (Marty et al. 2013).

Badawi et al. (2000) reported that gavage administration of a single dose of 375 mg 2,4-D/kg to rats
induced the expression of cytochromes CYP1A1, CYP1A2, and CYP1B1, which resulted in increased
metabolism of estrogen in liver, kidney, and mammary gland. It should be noted, however, that
375 mg/kg is a high dose of 2,4-D, at least half of the oral LD50 dose for rats, and is unlikely to be
encountered in environmental exposures to 2,4-D.

2,4-D did not bind to the androgen receptor (AR) in an in vitro AR bindings assay using a recombinant rat
AR (Fang et al. 2003). 2,4-D showed no estrogenic or anti-estrogenic activity in a two hybrid assay
system or anti-estrogenic activity in a reporter gene assay system using MCF-7 cells (Jung et al. 2004;
Nishihara et al. 2000). 2,4-D did not show estrogenicity in other studies using MCF-7 breast cancer cells
(Lin and Garry 2000; Soto et al. 1995). 2,4-D did not show estrogenic activity in a competitive-binding
assay utilizing estrogen receptor from uteri from ovariectomized rats (Blair et al. 2000). Orton et al.
(2009) reported that 2,4-D did not exhibit estrogenic, androgenic, anti-estrogenic, or anti-androgenic
activity in a recombinant yeast assay in vitro at environmentally relevant concentrations. Similar negative
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results were shown in an in vitro reporter gene assay using Chinese hamster ovary cells (Kojima et al. 2004). 2,4-D did not show androgenic activity in human prostate cancer cells in vitro and had no significant effect on either mRNA or protein levels of AR; however, 2,4-D with 5α-dihydroxytestosterone showed synergistic androgenic activity through, in part, the promotion of AR nuclear translocation (Kim et al. 2005).

The EPA recently completed a weight-of-evidence analysis of the potential interaction of 2,4-D with the androgen, estrogen, and thyroid signaling pathways and concluded that there is no convincing evidence of interaction with either of the three pathways (EPA 2015c, 2015d). Specifically, results from an in vitro AR binding assay using rat prostate cytosol showed that 2,4-D was negative for AR binding at concentrations up to $10^{-4}$ M. In an in vitro aromatase assay, aromatase activity for 2,4-D was similar to full activity controls at all concentrations of 2,4-D tested. The results from an ER binding assay using rat uterine cytosol showed that 2,4-D was negative for ER binding at concentrations of up to $10^{-4}$ M. Results from an in vitro estrogen receptor transcriptional activation assay in a human cell line indicated that 2,4-D treatment did not result in ER-mediated transcriptional activation at any concentration relevant for use in the assay. In an in vitro steroidogenesis assay using human adrenocortical carcinoma cells, 2,4-D treatment produced a statistically significant increase in estradiol production at the assay limit-concentration of $10^{-4}$ M. Because the increase in estradiol production did not meet the 1.5-fold cut off established in the validation program for the assay, it was not considered biologically relevant. 2,4-D did not significantly affect testosterone production at any concentration tested.

3.7 CHILDREN’S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when most biological systems will have fully developed. 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. Relevant animal and in vitro models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children’s unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.
Children sometimes differ from adults in their susceptibility to adverse health effects from exposure to hazardous chemicals, but whether there is a difference depends on the chemical(s) (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to exposure-related health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life that are most sensitive to disruption from exposure to hazardous substances. Damage from exposure in one stage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). Past literature has often described the fetus/infant as having an immature (developing) blood-brain barrier that is leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the brain, there are differences between fetuses/infants and adults that are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; whether these mechanisms provide protection for the developing brain or render it more vulnerable to
3. HEALTH EFFECTS

toxic injury is an important toxicological question. Chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns given their low glomerular filtration rate and not having developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

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 parents 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. 2013; 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 3.2.2.6, 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).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of a generalizable sample of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data
for 2,4-D from this report is discussed in Section 6.5. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 2,4-D are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 2,4-D are discussed in Section 3.8.2.

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 3.10, Populations That Are Unusually Susceptible.

### 3.8.1 Biomarkers Used to Identify or Quantify Exposure to 2,4-D

As mentioned in Section 3.3, 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 (see Chapter 7), 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 6.

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 (see Section 3.11.2).
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 the National Health and Nutrition Examination Survey (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 et al. 2010; 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 below exposure guidance values for 2,4-D.

3.8.2 Biomarkers Used to Characterize Effects Caused by 2,4-D

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.

3.9 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.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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. Populations who are at greater risk due to their
unusually high exposure to 2,4-D are discussed in Section 6.7, Populations with Potentially High Exposures.

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).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 2,4-D. Because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 2,4-D. When specific exposures have occurred, poison control centers, board certified medical toxicologists, board-certified occupational medicine physicians and/or other medical specialists with expertise and experience treating patients overexposed to 2,4-D can be consulted for medical advice. The following texts provide specific information about treatment following exposures to 2,4-D:


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Additional relevant information can be found in the front section of this profile under QUICK REFERENCE FOR HEALTH CARE PROVIDERS.

3.11.1 Reducing Peak Absorption Following Exposure

The following information was extracted from the books listed above; specific chapters were written by Roberts (2015), Bradberry et al. (2007), and Craig (1998). It is recommended, however, that this information be used along with consultation with a medical specialist with expertise and experience in treating/managing patients with phenoxy herbicide poisoning.

No information specific for 2,4-D was located; however, Roberts (2015) stated that oral activated charcoal may be given if the patient presents within 1–2 hours of ingestion of an herbicide known to cause significant poisoning. Administration of 50–100 g to an adult may be considered in severely poisoned patients within 1 hour of ingestion (Bradberry et al. 2007). In cases of dermal contact, hair and skin should be cleansed to prevent skin absorption (Craig 1998).

3.11.2 Reducing Body Burden

The following information was extracted from the books listed above; specific chapters were written by Roberts (2015), Bradberry et al. (2007), and Craig (1998). It is recommended, however, that this information be used along with consultation with a medical specialist with expertise and experience in treating/managing patients with phenoxy herbicide poisoning.

Management of patients with acute intoxication with 2,4-D is mainly supportive. However, patients with significant poisoning should be monitored for 24–48 hours, preferably in an intensive care unit (Roberts 2015). Patients with severe hypotonia may be unable to use intercostal muscles for ventilation and would benefit from a period of positive pressure mechanical ventilation (Craig 1998). Because 2,4-D is eliminated almost exclusively in the urine, an adequate renal output may optimize renal excretion and reduce renal toxicity from rhabdomyolysis (Roberts 2015). Urinary alkalization and hemodialysis should be considered in cases of severe poisoning. Increasing urine pH increases clearance of phenoxy herbicides by “ion trapping” of the chemicals. In one case, increasing urine pH from 5.0 to 8.0 increased renal clearance of 2,4-D from 5.1 to 63 mL/minute (Roberts 2015). It was noted that plasma
alkalinization also may limit the distribution of phenoxy compounds from the central circulation by “ion trapping.” Roberts (2015) also noted that “Because phenoxy compounds are small and water soluble, and subject to saturable protein binding with large exposures (increasing the free concentration), they are likely to be cleared by extracorporeal techniques. Extracorporeal elimination using resin hemoperfusion, hemodialysis, or plasmapheresis has been studied in a few cases, with clearances approaching 75 mL/minute.” Hemodialysis, however, is the preferred treatment in all severe cases because it greatly enhances clearance without the need for urine pH manipulation and the administration of considerable amounts of intravenous fluid to compromised patients (Bradberry et al. 2007).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism(s) of toxic effects of 2,4-D have not been clearly established; therefore, there are no established methods to interfere with the toxic effects of 2,4-D.

3.12 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 the National Toxicology Program (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.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health 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.

3.12.1 Existing Information on Health Effects of 2,4-D

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-D are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of 2,4-D. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the
### Figure 3-5. Existing Information on Health Effects of 2,4-D

#### Human

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<th>Method</th>
<th>Death</th>
<th>Acute</th>
<th>Intermediate</th>
<th>Chronic</th>
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<th>Neurologic</th>
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#### Animal

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● Existing Studies

***DRAFT FOR PUBLIC COMMENT***
quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 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.

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.

The database in animals is extensive. As can be seen in Figure 3-5, 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.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. 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
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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 end points 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). An intermediate-duration oral MRL based on developmental effects in rats (Stürtz et al. 2010) was adopted also as acute-duration oral MRL for 2,4-D. 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 Exposure. 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 Exposure and Cancer 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, 1996c; EPA 1984, 1985, 1986, 1987b; Gorzinski et al. 1987; Marty et al. 2013; Mattsson et al. 1997; Mazhar et al. 2014; Ozaki et al. 2001; Saghir et al. 2013; 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. 2013; Stürtz et al. 2010; Troudi et al. 2012a, 2012b). These studies suggested that the kidney is a target for 2,4-D toxicity. However, because of inconsistencies between studies and uncertainties regarding the toxicological significance of the renal lesions described in some of the reports available for review, this information was not considered for MRL derivation. It would be helpful if the original studies could be obtained to clarify these issues. However, data from a developmental study in rats (Stürtz et al. 2010) were used 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 end points in rats exposed to 2,4-D dusts for 28 days and established a LOAEL of 50 mg/m³.
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 Exposure and Cancer.** 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. 2006; Klucina 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.

Few chronic-duration studies in animals were available for review. These studies provided information on a wide range of end points 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. 1996b; EPA 1987a; Hansen et al. 1971). Results from the 2-year study in mice by Charles et al. (1996b) were considered for derivation of a chronic-duration oral MRL for 2,4-D. However, this resulted in a chronic-duration oral MRL for 2,4-D greater than the intermediate-duration oral MRL. Therefore, a chronic-duration oral MRL for 2,4-D was not derived. 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.

**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
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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. 1999a; 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; Gonzales 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. Studies of this nature are important in establishing a link between metabolism, DNA damage, and potential cancer(s).

Reproductive Toxicity. 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, 1996c; EPA 1984, 1985, 1986, 1987a; Gorzinski et al. 19897; 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. 2013). 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) though some studies describe behavioral effects (Bortolozzi et al. 1998, 1999, 2003; Evangelista de Duffard et al. 1995).

Developmental Toxicity. 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 end points 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. A study reported a relatively low LOAEL of 2.5 mg 2,4-D/kg/day for reduced body weight in 10-day-old rat pups from dams exposed to 2,4-D on postpartum days 1–16 (Stürtz et al. 2010). This study was used to derive an intermediate-duration oral MRL for 2,4-D. It would be reassuring if other groups of investigators can replicate the findings of Stürtz et al. (2010). Although, as mentioned above, no adverse health outcomes have been reported in children whose mothers were exposed to 2,4-D through farming activities, no information is available regarding levels of 2,4-D in breast milk or in neonates born to these women; pertinent studies would provide useful data.

**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. 1996a, 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. 2006; Tanner et al. 2009). Only Tanner et al. (2009) reported a positive 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. 1996a 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). A study identified a relatively low LOAEL of 15 mg 2,4-D/kg/day for altered maternal behavior in rats dosed on postpartum days 1–6 (Stürtz et al. 2008). However, the relevance of the alterations (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) to human health is unknown. The available chronic-duration oral studies did not conduct neurobehavioral tests. Considering that humans may be exposed to low levels of 2,4-D in food items or in drinking water, it would be valuable to determine whether prolonged, low-level exposure to 2,4-D may induce neurobehavioral alterations.

**Epidemiological and Human Dosimetry Studies.** Many epidemiological studies provided information regarding exposure to 2,4-D and a wide range health outcomes (see Chronic-Duration Exposure and Cancer 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.
Methods for Reducing Toxic Effects. There are no 2,4-D-specific effects following exposure to this chemical. Overexposure to 2,4-D has been associated with tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects. The mechanisms by which these effects occur have not been elucidated. Management of suspected 2,4-D related toxicity is essentially supportive. Information is available regarding methods that can be used to reduce toxic effects of phenoxy herbicides in general, including gastrointestinal decontamination, hemodialysis, and urinary alkalinization (Bradbury 2007; Roberts 2015). Publishing treatments that have proved to be effective in randomized controlled trials in medical journals could improve and/or prevent secondary effects and speed recovery in the most severe cases.

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. 2013; Sandberg et al. 1996; Stürtz et al. 2000, 2006). Although there are no reports of 2,4-D in human breast milk, monitoring of women with the highest exposures in farming communities would provide valuable information.

As summarized in Section 3.2.2.6, 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.
Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

### 3.12.3 Ongoing Studies

The following ongoing research pertaining to 2,4-D was identified in the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2015):

Atin Adhikari, from the University of Cincinnati, and coworkers are investigating the potential association between exposure to pesticides (2,4-D among them) among participants in the AHS and atopic immune responses. In the first phase of the research, the investigator will explore immunological activities of unpurified but clinically relevant environmental samples collected in farms (before and after pesticide application) in ovalbumin allergen sensitized mice. The study is sponsored by the National Institute of Environmental Health Sciences (NIEHS).

Laura Beane Freeman, from the Division of Cancer Epidemiology and Genetics of the National Cancer Institute (NCI), and coworkers are investigating potential associations between exposure to pesticides (2,4-D among them) and a wide range of health end points in participants in the AHS. Health end points evaluated include numerous types of cancer, noncancer conditions, and biologic measures. The research is sponsored by the NCI.

Dale Sandler, from the NIEHS, and coworkers are investigating potential associations between exposure to pesticides (2,4-D among them) and health end points among participants in the AHS. The primary focus of the current research is identifying incident cases of respiratory, neurologic, and autoimmune diseases as well as other outcomes reflecting the older age of the cohort. The research is sponsored by the NCI, NIEHS, and EPA.