2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 2,4-D IN THE UNITED STATES

2,4-D is a free acid pesticide widely used in the United States (see Table 4-1). While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D. Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester. Almost 90–95% of total global use is accounted for by dimethyl amine salt and ethylhexyl ester. 2,4-D and its different chemical forms are listed as an ingredient, either as the singular active ingredient or in conjunction with other ingredients, in about 600 agricultural and residential products. 2,4-D is one of the most widely used agricultural herbicides in the United States with approximately 39 million pounds applied to crops in 2013, with pasture and hay fields and wheat, soybean, and corn crops receiving the greatest applications. It is also applied to residential or commercial turf for the elimination of a wide variety of broadleaf weeds without causing harm to the grass.

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

The general population may be exposed to 2,4-D during and after its use in residential and recreational areas. 2,4-D applications often occur to residential lawns, golf courses, parks, cemeteries, and other grassy areas. Since 2,4-D is also used on aquatic weeds, swimmers may also be exposed. 2,4-D can unintentionally be transported into residences if clothing or shoes containing this substance are worn indoors or if pets track in 2,4-D from recently treated lawns. The general population can be exposed to 2,4-D by ingesting food or water contaminated with it or through dermal contact with it when used in residential settings (lawn applications). Populations living within or very near areas of heavy agricultural
2.4-D use have an increased risk of exposure to relatively larger amounts of 2,4-D through dermal contact with contaminated plants, soils, or surface waters or by inhalation of the mist formed from the applied herbicide. Those likely to receive the highest exposures are those who are involved in the production, formulation, handling, and application of 2,4-D. Dermal contact appears to be the major route of exposure for workers, although inhalation exposure and accidental ingestion via hand-to-mouth activity is possible.

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

### 2.2 SUMMARY OF HEALTH EFFECTS

Information regarding health effects in humans following exposure to 2,4-D comes from case reports of accidental or intentional ingestion of herbicide formulations containing 2,4-D, accidental skin contact with those products by farmers and professional residential applicators and homeowners (see Section 3.2.3, Dermal Exposure, for multiple references), and occupational exposure during manufacture. Effects that have been reported following oral or dermal exposure to high amounts of 2,4-D include tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects characterized by sensory and motor abnormalities. In two reports of dermal exposure, signs and symptoms of peripheral neuropathy persisted for a long time. Some of these studies estimated exposure levels and/or measured levels of 2,4-D in the body. A report estimated an ingested dose of approximately 80 mg/kg in a fatal case. In another fatal case, the investigators estimated that the subject had ingested at least 25–35 g of 2,4-D (357–500 mg/kg for a 70 kg body weight). However, there is a report of two individuals who survived after ingesting approximately 40 and 140 g of 2,4-D (571 and 2,000 mg/kg) in herbicide products. It should be kept in mind that these numbers are the result of the combined action of 2,4-D and other substances in the commercial formulations. In addition, whether or not deaths occurred may be related to the time elapsed between poisoning and beginning of emergency medical treatment.
Numerous epidemiological studies, mostly case-control and cohort studies, have examined potential associations between exposure to 2,4-D and multiple health outcomes including respiratory effects, endocrine effects, ocular effects, body weight effects, immunological effects, neurological effects, reproductive effects, developmental effects, various cancers (see Section 3.2.3.7, Cancer, for multiple references), and death.

While some of the human studies reported significant associations between use/exposure to 2,4-D and adverse health outcomes, some did not. It should be kept in mind also that pesticide applicators and farm workers are likely to be exposed to multiple chemicals, and even if analyses can be conducted for exposures to individual chemicals, a significant association between use/exposure and increased prevalence of an adverse health outcome does not necessarily imply causality, although it suggests that exposure to the chemical plays a role in the health outcome assessed and that biological plausibility exists. In general, issues that limited the interpretation of both positive and negative associations reported included lack of relationship with frequency of use of 2,4-D or the amounts of 2,4-D used, duration of exposure, or too few cases reported for a meaningful interpretation.

Among the various types of cancers examined (lymphatic system cancers, gastrointestinal cancer, breast cancer, cancers of the nervous system, prostate cancer, and others), lymphatic system cancers, in particular non-Hodgkin’s lymphoma (NHL), has received the most attention and has been the subject of several reviews. Some case-control studies reported that exposure to 2,4-D increased the risk of NHL, but others did not. The latter included cases of agriculture exposure, residential use of 2,4-D, exposure during manufacture, or in children from parents participating in the Agricultural Health Study (AHS). 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 funded by the National Cancer Institute and the National Institute of Environmental Health Sciences in collaboration with the EPA and NIOSH. No significant differences were reported in a few studies that assessed combinations of 2,4-D and other phenoxy acids such as 2,4,5-T or 2,4-dichlorophenoxypropionic acid (2,4-DP) and 2,4-dichlorophenoxybutyric acid (2,4-DB). Studies that examined cause-specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-D and related salts did not find patterns suggestive of a causal association between exposure to 2,4-D and any particular cause of death, including NHL. Overall, 2,4-D has exhibited low toxicity in studies of humans environmentally or occupationally exposed to this chemical.
The database in animals is extensive and consists mostly of studies by the oral route of exposure. In the only inhalation study available, intermittent nose-only exposure of rats to 2,4-D dusts for 28 days resulted in relatively low systemic toxicity; however, the lowest concentration tested, 50 mg/m³, induced histological alterations in the larynx (portal-of entry effect). Oral studies in animals have reported a wide range of effects in acute-, intermediate-, and chronic-duration studies. Acute-duration studies have reported LD₅₀ values ranging from 100 mg/kg in dogs to 1,000 mg/kg in guinea pigs. Dogs appear to be more sensitive than rats and mice. This appears to be due to dogs having a significantly lower capacity to eliminate 2,4-D via the kidneys than other species, including humans. Systemic effects reported in repeated exposure oral studies include hematological alterations in rats (decreased hemoglobin, platelets, and erythrocyte counts); hepatic effects in rats (histological alterations) and dogs (perivascular inflammation); renal effects in rats, mice, and dogs; alterations in thyroid hormone levels in rats; ocular effects in rats; and alterations in body weight gain in most species tested. Some apparent inconsistent results between studies, particularly regarding hepatic, renal, and thyroid effects, make it difficult to make generalizations and define reliable no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs). For example, a 13-week study reported a LOAEL of approximately 7.1 mg 2,4-D/kg/day for histological lesions in the kidneys of rats. However, other 13-week or shorter duration studies in rats reported LOAELs for histological alterations in the kidneys only at doses ≥20 mg 2,4-D/kg/day. In another study, female rats exposed to ≥15 mg 2,4-D/kg/day for 27 weeks had significantly increased serum thyroxine (T₄), but no increase was evident after 52 weeks of exposure and no alterations were seen in males exposed to up to 45 mg 2,4-D/kg/day at either time point. In addition, the toxicological significance of the results from some studies is not clear, as is the case, for example, for alterations in the kidneys from rats and mice characterized as increased homogeneity of the cytoplasm and decreased vacuolization of cells in the renal cortex. Studies in animals suggest that the respiratory, gastrointestinal, and cardiovascular systems are not sensitive targets for 2,4-D toxicity.

Results from in vivo and in vitro studies showed no evidence that 2,4-D is an endocrine disruptor substance. 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 any of the three pathways.

Exposure to 2,4-D did not affect the gross or microscopic morphology of lymphoreticular organs and tissues of animals as shown in multiple studies. Oral exposure of rats to 2,4-D did not affect immunocompetence, assessed by the sheep red blood cell (SRBC) antibody plaque forming cell assay. 2,4-D was a respiratory allergen in mice following dermal sensitization and challenge with the chemical
intratracheally. This information is insufficient to draw conclusions regarding 2,4-D and the immune system.

In general, exposure to 2,4-D did not induce gross or microscopic alterations in tissues of the nervous system of animals, but a relatively high single dose of 150 mg 2,4-D/kg altered the blood brain barrier in rats leading to vascular damage in the central nervous system. Exposure to 2,4-D induced neurobehavioral alterations in some studies. Worth noting is a relatively low LOAEL of 15 mg 2,4-D/kg/day (the lowest dose tested) for altered maternal behavior in rats dosed on postpartum days 1–6. 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. Single high doses of 250 mg 2,4-D/kg altered gait and motor activity in rats, whereas repeated doses of ≥20 mg 2,4-D/kg/day increased grip strength. The available data suggest that 2,4-D is not a neurotoxic substance at environmentally relevant doses (in the low µg/kg body weight/day range). However, it is unknown whether neurobehavioral alterations could occur as a result of chronic-duration exposure to low doses of 2,4-D. Available chronic-duration studies did not conduct neurobehavioral tests.

Exposure of male and female animals to 2,4-D through the diet did not affect the morphology of reproductive organs, nor did it affect mating and fertility indices or sperm parameters. However, histological alterations in Sertoli and Leydig cells and reduced sperm count and motility were reported in rats administered ≥50 mg 2,4-D/kg/day by gavage for 30 days. There is no explanation for this apparent discrepancy in results regarding sperm parameters other than the different modes of administering 2,4-D to the animals (i.e., diet versus gavage). Studies with exposure routes relevant to general population exposures suggest that 2,4-D is not a reproductive toxicant.

Perinatal exposure to 2,4-D has resulted in developmental effects, mostly reduced fetal or offspring weight and minor soft-tissue and skeletal anomalies, in some studies, but it did not induce teratogenicity. In many cases, reduced fetal weight was accompanied by reduced maternal weight gain during pregnancy or some other maternal effect. A low LOAEL of 2.5 mg 2,4-D/kg/day was reported for reduced body weight in 10-day-old rat pups from dams exposed on postpartum days 1–16. The effect was attributed to inhibition of the suckling-induced hormone release milk transfer to the litter by an action of 2,4-D at the level of the central nervous system. Other studies have reported reduced offspring weight but at higher
maternal exposure levels. Other developmental effects reported include neurobehavioral alteration in rat pups and delayed vaginal opening at maternal doses of 70 mg 2,4-D/kg/day and histological alterations in rat pup liver and bone at maternal doses of 126 mg 2,4-D/kg/day. 2,4-D did not induce developmental effects in hamsters following maternal exposure to ≤100 mg 2,4-D/kg/day or in rabbits following maternal exposure to ≤90 mg 2,4-D/kg/day. With the exception of the relatively low LOAEL of 2.5 mg/kg/day for reduced offspring weight, 2,4-D does not appear to be a strong developmental toxicant.

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

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been established for 2,4-D. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

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

**Inhalation MRLs**

No inhalation MRLs were derived for 2,4-D. Only one inhalation study was available for review. In that study, male and female rats were exposed nose-only 6 hours/day, 5 days/week for 28 days to 2,4-D dusts in target concentrations of 0, 50, 100, 300, and 1,000 mg/m$^3$ (EPA 2008). After termination of exposure, controls and rats from the highest exposure concentration group were kept for a 4-week recovery period to assess reversibility of the effects. A significant reduction in reticulocytes occurred in males and females at ≥300 mg/m$^3$ 2,4-D and a significant increase in serum alkaline phosphatase was reported in females at ≥300 mg/m$^3$ 2,4-D. No significant histological alterations were reported in the tissues and organs examined. The most salient effect was the occurrence of squamous/squamoid epithelial metaplasia with hyperkeratosis in the larynx of all exposed groups, with increasing severity as the exposure concentration increased. The lesions persisted during the recovery period, but with reduced severity. Therefore, the exposure concentration of 50 mg/m$^3$ 2,4-D represents the study LOAEL, a portal-of-entry LOAEL. Although this is a well-conducted study that examined a comprehensive number of end points, the database is insufficient for MRL derivation. It would be important to determine a NOAEL for the portal-of-entry effects.

**Oral MRLs**

An acute-duration oral MRL for 2,4-D was not derived. However, it is recommended that the intermediate-duration oral of 0.009 mg 2,4-D/kg/day be adopted also as acute-duration oral MRL for 2,4-D based on the information discussed below.

No adequate acute human data were located. Information regarding health effects in humans following acute-duration exposure to 2,4-D is limited to case reports of intentional or accidental ingestion of herbicide formulations containing 2,4-D. Effects that have been reported following oral exposure to high amounts of 2,4-D include tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and death (Dudley and Thapar 1972; Durakovic et al. 1992; Keller et al.
While some of these studies provided estimates of amounts of 2,4-D ingested, as stated earlier, the reported effects represent the result of exposure to a chemical mixture consisting of 2,4-D and other substances present in the commercial formulations (i.e., solvents, other herbicides), which is the exposure that most humans experience. Yet, the common exposure reported across studies was to 2,4-D. Two studies with volunteers in which the subjects were administered a single gelatin capsule containing a dose of 5 mg/kg 2,4-D reported no ill effects among the volunteers during the 1-week monitoring period that followed dosing (Kohli et al. 1974; Sauerhoff et al. 1977). Without specifying, Sauerhoff et al. (1977) stated that no untoward effects were associated with ingestion of 2,4-D. Kohli et al. (1974) monitored blood pressure, heart rate, hemoglobin content, and total and differential white cell counts and stated that no significant changes were noted during the study. The available information in humans is inadequate for MRL derivation.

Studies in animals provide information on lethality and a wide range of end points. The lowest lowest-observed-adverse-effect level (LOAEL) in an acute-duration study was 15 mg 2,4-D/kg/day for behavioral alterations and decreased serum prolactin levels in rats (Stürtz et al. 2008). In that study, rats were administered 2,4-D mixed in the food on postpartum days 1–7. During this time, specific maternal behaviors were monitored and quantified. After the last observation period, the rats were killed and blood was collected for analysis of prolactin. The brain was removed and endogenous monoamines were determined in the arcuate nucleus. The study reported that exposure to ≥15 mg 2,4-D/kg/day (lowest dose tested) significantly 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. In addition exposure to 2,4-D significantly decreased serum prolactin levels compared to controls. Biochemical analyses of the arcuate nucleus showed decreased serotonin at ≥15 mg/kg/day and increased dopamine at ≥25 mg/kg/day. Information regarding the body weight of the pups was not provided.

Long-term oral studies suggest that the kidney is a target for 2,4-D toxicity; however, only one acute-duration study conducted microscopic examinations of the kidneys. Steiss et al. (1987) reported no significant histological alterations in the kidneys from dogs dosed once with 125 mg 2,4-D/kg in a capsule (highest dose tested). Two studies defined LOAELs of 50 mg/kg/day. In one of these studies, doses of 50 mg 2,4-D/kg/day (lowest dose tested) induced significant weight loss in pregnant Wistar rats when administered by gavage in water on gestation days (GDs) 6–15 (Fofana et al. 2000). It is not totally clear, however, whether the investigators meant that the final weight was lower than the starting weight or whether treated rats just gained less weight than control rats. In another developmental study,
administration of 50 mg 2,4-D/kg/day by gavage in corn oil to pregnant Sprague-Dawley rats also on GDs 6–15 did not affect maternal weight (terminal weight similar in treated and controls), but induced a statistically significant reduction in fetal weight (approximately 7%) measured on GD 20 and increased the incidence of some soft-tissue anomalies and skeletal malformations; the NOAEL was 25 mg 2,4-D/kg/day (Schwetz et al. 1971).

Data from Stürtz et al. (2008) could be considered for MRL derivation, specifically, the reduction in maternal serum prolactin levels or some of the altered maternal behaviors. For both end points, the lowest dose of 2,4-D tested, 15 mg/kg/day, was a LOAEL. However, in a subsequent study, the same group of investigators reported that exposure of adult rats to dietary doses of 2,4-D ranging from 2.5 to 70 mg/kg/day on postpartum days 1–16 resulted in significantly reduced pup body weight during the first 16 days of life (Stürtz et al. 2010). Statistically significant differences with controls were seen beginning on postnatal day (PND) 7 at maternal doses ≥5 mg/kg/day. From PND 10 on, even the lowest maternal dose of 2,4-D tested, 2.5 mg/kg/day, induced a significant reduction in pup weight relative to the control group, thus making this dose level a LOAEL for acute-exposure duration. Because the various data sets for body weight changes on PNDs 10–14 did not show clear dose-response relationships, attempts to derive an acute-duration oral MRL from any one of these data sets using benchmark dose (BMD) analysis were unsuccessful. Therefore, it is recommended that the intermediate-duration oral MRL of 0.009 mg 2,4-D/kg/day, which was derived by performing BMD analysis of the pup body weight data for PND 16 (see below), also be adopted as acute-duration oral MRL for 2,4-D.

- An MRL of 0.009 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,4-D.

The MRL is based on a BMDL_{RD05} (benchmark dose lower bound, 5% change from control) of 0.93 mg 2,4-D/kg/day for decreased body weight in rat’s offspring on PND 16 (Stürtz et al. 2010). No human data were located. The database for animals is extensive and suggests that the kidney is a target organ for 2,4-D toxicity. Dogs appeared to be more sensitive than rodents, and as mentioned earlier, this seems due to dogs having a significantly lower capacity to eliminate 2,4-D via the kidneys than other species, including humans (Timchalk 2004). Therefore, dogs might not be a relevant species for evaluation of human health risk, and will not be considered for MRL derivation. The lowest LOAEL identified among intermediate-duration oral studies is 2.5 mg 2,4-D/kg/day for alterations in milk ejection in rat dams and reduced postnatal pup weight during maternal exposure to 2,4-D in the food on postpartum days 1–16 (Stürtz et al. 2010). Reduced offspring body weight was also reported in other studies in which rat dams were exposed to 2,4-D for longer periods that also included postpartum, although at higher estimated
maternal doses. In a 2-generation reproductive study, pup body weight was reduced significantly on PND 28 at estimated maternal doses ≥35 mg 2,4-D/kg/day during lactation, but not at 10 mg 2,4-D/kg/day (EPA 1986). Marty et al. (2013) reported significantly reduced pup weight (about 10%) on PND 22 at estimated maternal doses of approximately 9 mg 2,4-D/kg/day during lactation, but lower doses were not tested. In a 3-generation study, reduced pup weight was noted at maternal doses of approximately 111 mg 2,4-D/kg/day, but not 37 mg/kg/day (Hansen et al. 1971). The reasons for the apparent discrepancy regarding maternal dose levels at which offspring weight is significantly affected are not clear, but could be related to the different manners of estimating maternal intake of test material.

Several studies reported histological alterations in the kidneys from rats following exposure to 2,4-D; the results are summarized in Table 2-1. As the table shows, there is considerable dispersion of the data. The lowest dose at which alterations were reported (as described in the report reviewed) is 5 mg 2,4-D/kg/day (incidence significantly different from controls) for increased brown pigmentation in tubular cells in male and female F-344 rats (EPA 1985). Degenerative changes were reported at 45 and 60 mg 2,4-D/kg/day in male and female F-344 rats (EPA 1984; Gorzinski et al. 1987) and at 25 and 45 mg 2,4-D/kg/day in male Sprague-Dawley rats (Marty et al. 2013; Saghir et al. 2013). Simple hyperplasia was reported in male Sprague-Dawley rats dosed with approximately 7.1 mg 2,4-D/kg/day for 13 weeks (Ozaki et al. 2001). In another 13-week study, Charles et al. (1996a) stated that histological alterations were seen predominantly at 300 mg acid equivalents/kg/day and consisted of brush border loss in proximal tubular cells and vacuolization of kidney tubular cells in both male and female F-344 rats, suggesting that the NOAEL was 100 mg/kg/day. However, no kidney lesions were listed for 2,4-D acid in Table 1 of the study, suggesting that none occurred or the incidence in the treated groups was not significantly different than in the control group.

In B6C3F1 mice, exposure to ≥15 mg 2,4-D/kg/day for 13 weeks resulted in increased incidence of homogeneity and altered tinctorial properties of the cytoplasm of renal epithelial cells and decreased intracellular/intraluminal vacuolization in the kidney cortex of males (EPA 1984). It is unclear whether these changes were adverse or not. The same alterations were reported in male B6C3F1 mice exposed to ≥15 mg 2,4-D/kg/day for 52 weeks (EPA 1987a). In yet another 13-week study in B6C3F1 mice, exposure to approximately 430 mg 2,4-D/kg/day (highest dose tested) caused lesions in renal tubular epithelial cells characterized as simple hyperplasia; no changes were reported at approximately 180 mg 2,4-D/kg/day (Ozaki et al. 2001).
### Table 2-1. Histological Alterations in Kidneys from Rats and Mice in Intermediate-Duration Studies

<table>
<thead>
<tr>
<th>Study details</th>
<th>LOAEL (mg/kg/day)</th>
<th>NOAEL (mg/kg/day)</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-344 rats, 13 weeks</td>
<td>300</td>
<td>100</td>
<td>Brush border loss in proximal tubular cells; vacuolization of kidney tubular cells (both sexes)</td>
<td>Charles et al. 1996a</td>
</tr>
<tr>
<td>F-344 rats, 13 weeks</td>
<td>45</td>
<td>15</td>
<td>Degenerative changes in kidneys (both sexes)</td>
<td>EPA 1984</td>
</tr>
<tr>
<td>F-344 rats, 52 weeks</td>
<td>5</td>
<td>1</td>
<td>Increased tubular cell (brown pigment) (both sexes)</td>
<td>EPA 1985</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>Moderate fine vacuolization of cytoplasm of renal cortex (females)</td>
<td></td>
</tr>
<tr>
<td>F-344 rats, 13 weeks</td>
<td>60</td>
<td>15</td>
<td>Slight multifocal degeneration of descending proximal tubules (both sexes)</td>
<td>Gorzinski et al. 1987</td>
</tr>
<tr>
<td>Sprague-Dawley rats, 2-generation</td>
<td>45</td>
<td>17</td>
<td>Slight degeneration of proximal convoluted tubules (F0 males)</td>
<td>Marty et al. 2013</td>
</tr>
<tr>
<td>Sprague-Dawley rats, 13 weeks</td>
<td>7.1</td>
<td>1.5</td>
<td>Simple hyperplasia (males)</td>
<td>Ozaki et al. 2001</td>
</tr>
<tr>
<td>F-344 rats, 2-generation</td>
<td>20</td>
<td>5</td>
<td>Increased focal nuclear density in medullary tubules (males)</td>
<td>EPA 1987b</td>
</tr>
<tr>
<td>Sprague-Dawley rats, 70 days</td>
<td>25</td>
<td>6</td>
<td>Slight degenerative multifocal lesions in proximal convoluted tubules (males)</td>
<td>Saghir et al. 2013</td>
</tr>
<tr>
<td>B6C3F1 mice, 13 weeks</td>
<td>15</td>
<td>5</td>
<td>Increased homogeneity and altered tinctorial properties of cytoplasm; decreased intracellular vacuolization in cortex (males)</td>
<td>EPA 1984</td>
</tr>
<tr>
<td>B6C3F1 mice, 52 weeks</td>
<td>15</td>
<td>1</td>
<td>Increased cytoplasmic homogeneity; decreased cytoplasmic vacuolization in tubular epithelium (males)</td>
<td>EPA 1987a</td>
</tr>
<tr>
<td>B6C3F1 mice, 13 weeks</td>
<td>430</td>
<td>179</td>
<td>Simple hyperplasia (males)</td>
<td>Ozaki et al. 2001</td>
</tr>
</tbody>
</table>

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level
Because of the inconsistencies between studies and the uncertainty in determining whether or not certain histological alterations in the kidneys from rats and mice should be considered adverse, these data were not considered for MRL derivation. Instead, data for body weight changes in rat offspring exposed to 2,4-D through maternal milk reported in the Stürtz et al. (2010) study were selected for derivation of an intermediate-duration oral MRL for 2,4-D.

As previously mentioned, in the Stürtz et al. (2010) study, groups of female Wistar rats (6–8/group) were fed a diet that provided 0, 2.5, 5, 10, 15, 25, 50, or 70 mg/kg/day 2,4-D (98% pure) on postpartum days 1–16. Dams were checked daily for clinical signs and food consumption and body weight were monitored. Milk ejection was assessed by changes in body weight of the pups after allowing the pups to suckle during 15-minute periods on postpartum days 11–13. Blood was collected on postpartum day 12 for determination of growth hormone, prolactin, and oxytocin. Dams were sacrificed on postpartum day 16, and the arcuate nucleus and the anterior lobe of the pituitary were isolated for biochemical analyses of monoamines and metabolites in the 15, 25, and 50 mg/kg/day dose groups. Maternal exposure to 2,4-D did not affect maternal body weight, and no pups died during the test period. Maternal exposure to 2,4-D significantly reduced pup weight beginning on PND 7 in all exposed groups except the lowest dose group; this group showed a significant reduction in body weight beginning on PND 10. Milk ejection was significantly reduced in all treated groups on postpartum day 13 by >50%, reaching approximately 75% reduction in the highest dose group. An injection of oxytocin to the dams partially restored milk production, indicating that 2,4-D, at least in part, inhibited oxytocin release, but not the capacity of the mammary gland to produce or secrete milk. Serum prolactin appeared to be reduced in all treated groups, although Figure 3A in the study does not indicate statistically significant differences between the controls and exposed groups. Serum oxytocin was significantly reduced at ≥25 mg 2,4-D/kg/day. Serotonin was significantly reduced in the arcuate nucleus at ≥15 mg 2,4-D/kg/day and dopamine was significantly increased at ≥25 mg/kg/day. Dopamine was also increased in the anterior pituitary at ≥15 mg 2,4-D/kg/day.

The offspring body weight data on PND16 were fit to all available continuous models in EPA’s Benchmark Dose Software (BMDS, version 2.4.0) using a benchmark response (BMR) of 5% change from control. Although there are no established guidelines as to what minimal change in a continuous end point such as body weight is biologically significant, a 10% change is generally used for adult body weight. However, because fetal or neonatal organisms may be more susceptible than adults, a 5% change was deemed appropriate.
Because no models fit the complete dataset, first the highest dose and subsequently the next highest dose were dropped. Only two BMD models (Exponential model 4 and Hill model) provided an adequate fit by the various statistical criteria. Because the BMDL_{RD05} estimates were sufficiently close, the model with the lowest Akaike’s Information Criterion (AIC) (Exponential model 4) was selected. The Exponential model calculated BMD_{RD05} and BMDL_{RD05} values of 1.27 and 0.93 mg 2,4-D/kg/day, respectively, for decreased pup body weight on PND 16. Dividing the BMDL_{RD05} of 0.93 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) yields an intermediate-duration oral MRL of 0.009 mg/kg/day for 2,4-D. Further details of the MRL derivation are presented in Appendix A.

A chronic-duration oral MRL for 2,4-D was not derived, as explained below. No adequate human data were located. A limited number of chronic-duration oral studies in rats, mice, and dogs were available for review. These studies suggest that the kidney is a target for 2,4-D toxicity in mice. As noted earlier, dogs might not be a relevant species for evaluation of human health risk due to their significantly lower capacity to eliminate 2,4-D via the kidneys; thus, dogs were not considered a suitable species for MRL derivation (see Section 3.5.1). A 2-year bioassay in F-344 rats defined an overall NOAEL of 5 mg 2,4-D/kg/day for organs and tissue histopathology and hematological and clinical chemistry parameters (Charles et al. 1996b). Exposure to 75 mg 2,4-D/kg/day decreased platelet and erythrocyte counts and hematocrit in females (results not shown), increased serum alanine aminotransferase (ALT) in males and decreased serum T4 in both sexes. Histological alterations were noted at 150 mg 2,4-D/kg/day and consisted of a nonsignificant increase in parafollicular cell nodular hyperplasia in the thyroid from females and minimal panlobular tinctorial properties in the liver from males and females. No clear treatment-related histological alterations were observed in the kidneys. An earlier study did not report treatment-related alterations in organs and tissues from Osborne-Mendel rats dosed with up to approximately 92 mg 2,4-D/kg/day in the diet for 2 years (Hansen et al. 1971).

In B6C3F1 mice, exposure to 15 mg 2,4-D/kg/day for 2 years significantly increased the incidence of cytoplasmic homogeneity in the renal tubular epithelium from male mice; this was attributed to a reduction of cytoplasmic vacuoles normally present in the cytoplasm of epithelial cells (EPA 1987a). No significant increase was seen at 1 mg 2,4-D/kg/day. The same alterations were observed in the kidneys from male B6C3F1 mice dosed with ≥62.5 mg 2,4-D/kg/day in another 2-year study (Charles et al. 1996b); no significant increase occurred at 5 mg 2,4-D/kg/day. A significant increase in minimal degeneration with regeneration of the descending portion of the proximal tubules in male mice occurred with an incidence of 0/50 (control), 0/50 (5 mg/kg/day), 25/50 (62.5 mg/kg/day), and 48/50

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(125 mg/kg/day), defining a NOAEL of 5 mg 2,4-D/kg/day and a LOAEL of 62.5 mg 2,4-D/kg/day for renal effects in this study (Charles et al. 1996b). No other treatment-related histological alterations in organs or tissues or in hematology tests were reported in mice in these studies. Because of the unclear biological significance of the reduced vacuolization of the cytoplasm in tubular cells in male B6C3F1 mice, the degeneration/regeneration change in the proximal tubule of male mice reported by Charles et al. (1996b) seemed to be a more toxicologically relevant end point for MRL derivation.

The incidence data for degeneration with regeneration of the descending portion of the proximal tubules in male mice were analyzed using all available dichotomous models in the EPA BMDS (version 2.4.0) using the extra risk option. Adequate model fit was judged by three criteria: goodness-of-fit p-value (p>0.1), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL (95% lower confidence limit on the BMD) was selected as the point of departure (POD) when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest Akaike information criterion (AIC) was chosen. All models except the Multistage (1-degree) model provided an adequate fit to the dataset. The model selected based on the criteria mentioned above was the Multistage (2-degree) model, which defined a BMDL of 23.59 mg 2,4-D/kg/day and a BMDL of 16.66 mg 2,4-D/kg/day. Dividing the BMDL of 16.66 mg/kg/day by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) would yield a chronic-duration oral MRL of 0.02 mg/kg/day for 2,4-D. However, this value is higher than the intermediate-duration oral MRL of 0.009 mg/kg/day for 2,4-D. Therefore, it is recommended that a chronic-duration oral MRL for 2,4-D not be derived at this time. The intermediate-duration oral MRL is protective for chronic-duration exposure.