DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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Peer reviewers for the post-public comment draft of the Toxicological Profile for 2,4-Dichlorophenoxyacetic Acid (2,4-D) were:

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Comments provided by Peer Reviewer #1

General Comments

COMMENT 1: Regarding Section 1.2, the Reviewer comments "This section lacks structure. First, [a]n overview of the non-cancer and cancer effects is given with very high-level conclusions. Later, organ-specific effects and cancer effects are discussed. Grouping of the summaries for the effects into non-cancer and cancer sections, and then into human and animal data sub-sections in each section is recommended."

RESPONSE: *ATSDR thanks the Reviewer for the comment. Section 1.2 is consistent with ATSDR's guidance document; ATSDR will consider the Reviewer's comments in subsequent revisions of the guidance document.*

COMMENT 2: Regarding the animal database in Section 1.2, the Reviewer questions "Exposure to what form(s) of 2,4-D?"

RESPONSE: *Most animal studies employed 2,4-D with a purity >95%. The few studies that employed salts of 2,4-D did not find significant differences in toxicity. It seems unnecessary to separate out various forms of 2,4-D in this section.*

COMMENT 3: Regarding the following sentence in Section 1.2—*Dogs exhibit a significantly lower capacity to eliminate 2,4-D via the kidney than do other species, including humans (Timchalk 2004)*—the Reviewer comments "This level of detail and a reference is somewhat odd to be included in a summary"

RESPONSE: *Since dogs were not considered an appropriate species for extrapolating to humans, the text was revised to delete effects observed in dogs from the summary list in Section 1.2 and the referenced statement was deleted:*

> The database in animals is extensive and consists almost exclusively of studies that employed the oral route of exposure. Systemic effects reported in repeated exposure oral studies in animals include hematological alterations in rats (decreased hematocrit, platelets, and erythrocyte counts); hepatic effects in rats (histological alterations); renal effects in rats and mice; alterations in thyroid hormone levels in rats; and ocular effects in rats.

COMMENT 4: With regard to Figure 1-1, Health Effects in Animals Following Oral Exposure to 2,4-D, the Reviewer asks "Non-cancer?"

RESPONSE: *The statement in question was revised to the following:*

> As illustrated in Figure 1-1, the most sensitive noncancer effects of repeated-dose oral exposure to 2,4-D are kidney effects and developmental effects.

COMMENT 5: Regarding the cancer effects summary paragraph in Section 1.2, the Reviewer questions "Why no references in this section even though numerous references were included in sections on non-cancer effects?"

RESPONSE: *The text in Section 1.2 was revised to include some of the epidemiological study citations and to include the citations for the animal studies:*

2,4-D has been evaluated for possible associations with a variety of cancer types (lymphatic system cancers, gastrointestinal cancer, breast cancer, cancers of the nervous system, prostate cancer, and others) (e.g., Goodman et al. 2015; Flower et al. 2004; Hoar et al. 1986; Pahwa et al. 2006; Smith et al. 2017; see Section 2.19 for full list of citations).

The carcinogenicity of 2,4-D has been evaluated in a number of animal cancer bioassays; species evaluated include rats, mice, and dogs (Charles et al. 1996a; EPA 1996a, 1996b, 1987a; Hansen et al. 1971).

COMMENT 6 : Regarding the following sentence in the cancer effects summary in Section 1.2—*Cancer of the lymphatic system, particularly non-Hodgkin's lymphoma (NHL), has received the most attention and has been the subject of several reviews*—the Reviewer comments "Reviews carry little weight in epidemiology, meta-analyses do… why mention that there were "reviews" without noting what were the conclusions of those "reviews". Were those "reviews" systematic?"

RESPONSE: *The phrase "and has been the subject of several reviews" was deleted.*

Cancer of the lymphatic system, particularly non-Hodgkin's lymphoma (NHL), has received the most attention.

COMMENT 7: Regarding the sentence in the cancer effects summary in Section 1.2—*The latter included cases of agriculture exposure, residential use of 2,4-D, exposure during manufacture, or children from parents participating in the Agricultural Health Study (AHS)*—the Reviewer questions "What about "the former"? Appears to be a focus on the negative findings."

RESPONSE: *The statement "The latter included…" was revised to:*

The case-control studies included agriculture exposure, residential use of 2,4-D, exposure during manufacture, or children from parents participating in the Agricultural Health Study (AHS).

COMMENT 8: Regarding the sentence in the cancer effects summary in Section 1.2—*Under the conditions of these bioassays, 2,4-D was not considered carcinogenic to rats, mice, or dogs*—the Reviewer notes "Need to include doses tested and duration of exposures?"

RESPONSE: *This statement is intended to inform the reader of the general results from the animal carcinogenicity studies. More quantitative data are presented in Section 2.19. The statement "Under the conditions of these bioassays, 2,4-D was not considered carcinogenic to rats, mice, or dogs" was revised to:*

These animal cancer bioassays did not provide convincing evidence of 2,4-D carcinogenicity.

COMMENT 9: Regarding the sentence in the cancer effects summary in Section 1.2—*The U.S. Environmental Protection Agency (EPA) has assigned 2,4-D to carcinogenicity Group D, "not classifiable as to human carcinogenicity"* —the Reviewer questions "What is the reference for this?"

RESPONSE: *The citation "EPA 2005a" was added to the statement in Section 1.2:*

The U.S. Environmental Protection Agency (EPA 2005a) has assigned 2,4-D to carcinogenicity Group D, "not classifiable as to human carcinogenicity."

COMMENT 10: Regarding the following sentence in the cancer effects summary in Section 1.2—*The International Agency for Research on Cancer (IARC) recently classified 2,4-D as possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and limited evidence in experimental animals (IARC 2018)* —the Reviewer comments "This is in stark contrast with the conclusions in a preceding paragraph that "2,4-D was not considered carcinogenic to rats, mice, or dogs"… Does something need to be said why the conclusion is different from IARC (2018)?"

RESPONSE: *As noted in the Response to Comment 8, the referenced statement in Section 1.2 was deleted.*

COMMENT 11: With regard to Figure 1-2, the Reviewer questions "why not plot the NOAELs on this figure as well? The table that follows used NOAELs for MRL derivation so it looks odd not to show them in Figure 1-2"

RESPONSE: *Figure 1-2 is intended to inform the reader of the levels at which adverse effects begin to occur.*

COMMENT 12: Referring to Figure 1-2, the Reviewer comments "Also, Figure 1-2 doesn't contain an x-axis. This makes it not clear if the circles are drawn to scale or not. I also would suggest including LD50 range (oral LD50 range from 100 to 1000 mg/kg according to the information on page 58... "death" LOAEL in acute exposures appears to be less that an order of magnitude away from the chosen NOAEL which doesn't look too comforting of a "margin of safety"."

RESPONSE: *Figure 1-2 is intended to identify the lowest reliable LOAEL values for the four most sensitive endpoints. The circles are drawn on an x-axis; ATSDR opted to include the LOAEL value in the circle rather than include the x-axis in the figure. As discussed in Section 2.2, the LD_{50} of 100 mg/kg reported in the Drill and Hiratzka (1953) study was not considered reliable; thus, it was not included in Figure 1-2.*

COMMENT 13: Regarding the literature search boilerplate text in Section 2.1, the Reviewer comments "The field of environmental risk assessment has entered the era of "systematic review" and the Profile needs to be explicit about the nature of the "literature search" and whether it was systematic according to the current standards (e.g., as defined by the "IRIS Handbook")"

RESPONSE: *This Toxicological Profile for 2,4-D was under development prior to ATSDR's initiation of the systematic review process. ATSDR has instituted the systematic review process for future toxicological profiles and includes a detailed summary of the systematic review process in an appendix.*

COMMENT 14: Regarding the boilerplate text in Section 2.1 discussing levels of significant exposure (LSEs), no-observed-adverse-effect levels (NOAELs), and lowest-observed-adverse-effect levels (LOAELs), the Reviewer comments "There should be explicit citations here to such "guidelines" and "policies""

RESPONSE: *ATSDR is reviewing the boilerplate text for possible revisions to provide additional explanation of LSEs, NOAELs, and LOAELs or to point the reader to more detailed information.*

COMMENT 15: With regard to Figure 2-1, Overview of the Number of Studies Examining 2,4-D Health Effects, the Reviewer questions "Where is a list of the references for these studies? What were the search terms, databases searched, years, etc.? This needs to be synergized with the information in Appendix B."

RESPONSE: *Figure 2-1 is intended to inform the reader of the numbers of studies that evaluated particular toxicity endpoints. All of the studies represented in Figure 2-1 are discussed in Sections 2.2 through 2.19. Detailed information regarding search strategies, etc., is presented in Appendix B.*

COMMENT 16: Regarding Section 2.4, Respiratory, the reviewer states "Please consider changing the order of sentences in this paragraph. It is not clear why the funding source is mentioned in between of the results and methods…"

RESPONSE: *The paragraph in question in Section 2.4 was revised:*

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

COMMENT 17: Regarding the following sentence in Section 2.4, Respiratory—*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)*—the Reviewer comments "An odd term. Workers? Residents on a farm? Wives of the applicators?... Prior paragraph details males as "participants"."

RESPONSE: *The statement regarding the farm women was revised to note that the women were wives of pesticide applicators:*

> In a group of 583 farm women (wives of pesticide applicators) in the AHS, prevalence of self-reported history of doctor-diagnosed chronic bronchitis was associated with lifetime exposure to 2,4-D in models adjusted for age and state (OR 1.29; 95% CI 1.02–1.63) (Valcin et al. 2007).

COMMENT 18: Regarding the following sentence in Section 2.10, Renal—*In the cross-sectional study described above in Section 2.9 (Schreinemachers 2010), subjects with measurable urinary levels of 2,4-D had significantly higher levels of urinary creatinine than subjects with undetectable levels, but still within the normal range*—the Reviewer requests "Please provide at least some basic information on who were participants in this study"

RESPONSE: *The statement was revised:*

In the NHANES cross-sectional study of 727 participants described above in Section 2.9 (Schreinemachers 2010),…

COMMENT 19: Regarding the non-Hodgkin's lymphoma (NHL) subsection in Section 2.19, Cancer, the Reviewer comments "Please separate each study into its own paragraph for ease of distinguishing among studies."

RESPONSE: *The individual studies were separated into paragraphs.*

COMMENT 20: With regard to text in Section 2.19, Cancer, discussing associations between NHL and exposure to 2,4-D, the Reviewer notes "The description appears imbalanced. Positive studies provided some information on their cohort size and also list limitations. Negative studies are just listed as negative and the reader has no idea of what their cohorts and type of study were. Neither it is clear if they suffered from limitations."

RESPONSE: *ATSDR considers it important to provide brief study details to studies that reported positive associations between 2,4-D and NHL. However, such details for studies that did not provide positive associations do not appear necessary.*

COMMENT 21: Regarding text in Section 2.19, Cancer, discussing associations between NHL and exposure to 2,4-D, the Reviewer notes "Please add the positive association to the other positive studies"

RESPONSE: *Risk ratios are provided for all studies reporting positive associations between 2,4-D and NHL.*

COMMENT 22: Regarding text in Section 2.20, Genotoxicity, describing the genotoxicity data presented in tables, the Reviewer questions "Why "some"? How were studies selected??"

RESPONSE: *IARC (2018) provides an extensive summary of available studies that evaluated the genotoxicity of 2,4-D. ATSDR has provided a reasonable sample of results from the genotoxicity studies. The reader may consult IARC (2018) for more detailed information.*

COMMENT 23: With respect to Table 2-4, the Reviewer notes "All studies need to list highest ineffective dose or lowest effective dose. Otherwise, it is impossible to interpret the + or – listings"

RESPONSE: *Table 2-4 employs the standard ATSDR format for genotoxicity results.*

COMMENT 24: Regarding the *in vitro* exposure study description in Section 2.20, Genotoxicity, the Reviewer states "The wording here is highly misleading. What is meant is that some studies were negative. But some studies were positive (listed below)."

RESPONSE: *The statements in question were revised:*

In vitro studies with other mammalian cells have demonstrated somewhat mixed results. Positive results were reported for mutation, chromosomal aberrations, SCEs, DNA damage, and

morphological cell transformation in Chinese and Syrian hamster cell lines (Ahmed et al. 1977; Galloway et al. 1987; González et al. 2005; Maire et al. 2007). Negative results were reported for SCEs in Chinese hamster ovary cells (Linnainmaa 1984), unscheduled DNA synthesis in primary rat hepatocytes (Charles et al. 1999a), and morphological cell transformation in Syrian golden hamster cells (Mikalsen et al. 1990).

COMMENT 25: Referring to Section 2.21.1, Pharmacokinetic Mechanisms, the Reviewer questions "Why is this section separate from section 3 – Toxicokinetics?"

RESPONSE: *This section presents information regarding possible pharmacokinetic-based mechanisms involved in 2,4-D toxicity.*

COMMENT 26: Regarding Section 2.21.2, Mechanisms of Toxicity, the Reviewer comments "This section lacks structure and conclusions. It seems that oxidative stress is a plausible mechanism of toxicity. No conclusions are drawn from the data."

RESPONSE: *The mechanism(s) of 2,4-D toxicity has not been fully elucidated. Section 2.1.2 has been revised to begin with a brief introduction of available mechanistic data:*

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

COMMENT 27: Regarding description of the Bradberry et al. (2000) study in Section 2.21.2, Mechanisms of Toxicity, the Reviewer notes "Not clear how far "below" is the text from a review. Please use quotation marks to identify text from Bradberry et al 2000."

RESPONSE: *The information presented in the summary of the Bradberry et al. (2000) review is not a direct quote. The discussion of the modes of action proposed by Bradberry was moved to a single paragraph.*

COMMENT 28: Regarding the following sentence in Section 2.21.2, Mechanisms of Toxicity—*The formation of a choline ester that could act as a false transmitter would affect muscarinic and nicotinic synapses*—the Reviewer comments "Full citation is needed"

RESPONSE: *A citation was not added for this statement. The reader has been referred to references in the review of Bradberry et al. (2000) for more detailed information because information in the summary paragraph comes from numerous primary sources cited by Bradberry et al. (2000).*

COMMENT 29: Regarding the following sentence in Section 5.7, Populations with Potentially High Exposures—*Populations who are at greater risk due to their unusually high exposure to 2,4-D are discussed in Section 5.7, Populations with Potentially High Exposures*—the Reviewer notes "It is odd to include a circular reference to the same section…"

RESPONSE: *The statement in question was deleted.*

COMMENT 30: Regarding Figure 6-1, Summary of Existing Health Effects Studies on 2,4-D by Route and Endpoint, the Reviewer notes "Suggest breaking this figure down by acute/sub-/chronic studies. It will be evident that chronic studies are limited – hence recommendation of adding an extra UF for the database weakness in the chronic oral MRL."

RESPONSE: *Figure 6-1 is designed to inform the reader of the numbers of studies (human and animal) available by route of exposure, not duration. Figure 2-1 provides information on the distribution of studies across exposure durations and illustrates that only 7% of the available toxicity studies in experimental animals were chronic duration. ATSDR disagrees with the Reviewer the chronic-duration oral MLR needs an uncertainty factor to account for weakness in the chronic oral database. The chronic-duration toxicity of 2,4-D has been evaluated in two rat studies, two mouse studies, and one dog study.*

COMMENT 31: Regarding the following sentence in the subsection discussing chronic-duration Minimal Risk Levels (MRLs) in Section 6.2, Identification of Data Needs—*Chronic-duration studies in animals were available for review*—the Reviewer questions "Would be better to say: "A limited number of…"??? A very small dataset is included that contains only one adequately reported 2 year cancer bioassay"

RESPONSE: *The sentence in Section 6.2 (Chronic-Duration MRLs) was revised to note that a limited number of chronic-duration animal studies was available:*

A limited number of chronic-duration studies in animals was available for review.

COMMENT 32: Regarding the comparative toxicokinetics subsection in Section 6.2, Identification of Data Needs, the Reviewer questions "Why are studies of inter-individual variability in TK and/or TD identified as a limitation? This can be done using rodent population-based models…"

RESPONSE: *The statement was intended to point out that there are sex and species differences in the toxicokinetics of 2,4-D that are related to OAT1 activity. This is not considered a limitation. However, there may be human populations at increased risk of 2,4-D toxicity based on toxicokinetic parameters such as OAT1 activity level.*

COMMENT 33: Regarding the MRL summary for the acute-duration inhalation MRL, the Reviewer states "This reviewer agrees"

RESPONSE: *No response is necessary.*

COMMENT 34: Regarding the MRL summary for the intermediate-duration inhalation MRL, the Reviewer states "This reviewer agrees"

RESPONSE: *No response is necessary.*

COMMENT 33: Regarding the MRL summary for the chronic-duration inhalation MRL, the Reviewer states "This reviewer agrees"

RESPONSE: *No response is necessary.*

COMMENT 34: Regarding the MRL summary for the acute-duration oral MRL, the Reviewer states "This reviewer agrees"

RESPONSE: *No response is necessary.*

COMMENT 35: Regarding the MRL Worksheet for the intermediate-duration oral MRL, the Reviewer states "This reviewer agrees that studies of post-natal weight changes in offspring of mothers exposed to 2,4-D are difficult to interpret in terms of confounding factors and establishing a dose level to be used for MRL derivation.
The choice of renal effects is warranted as this is a tissue that is a target in all species studied, including human case studies of poisonings. TK also supports kidney as a target organ.
This reviewer would recommend selection of 5 mg/kg as a NOAEL for MRL derivation from rat and mouse 52-week studies (EPA 1984, EPA 1987, Charles et al. 1996a; EPA 1996a,b – 2,4-D) – see also below"

RESPONSE: *The NOAEL of 16.6 mg/kg/day (associated with a LOAEL of 45.3 mg/kg/day for increased kidney weight and increased incidence of histopathologic kidney lesions) from the study of Marty et al. (2013) was selected as the point of departure for deriving an intermediate-duration oral MRL for 2,4-D, rather than the results from EPA (1984, 1987a) because it included a more complete morphological description of the kidney lesions (including increased thickness of the basement membrane in the affected tubules). The 52-week study in rats (Charles et al. 1996a; EPA 1996a) only described the kidney lesions as degeneration in the proximal tubules; furthermore, the LOAEL of 75 mg/kg/day in this study is higher than the LOAEL of 45.3 mg/kg/day identified by Marty et al. (2013). Based on the contention that adverse kidney effects occur when the saturation limit for kidney clearance of 2,4-D is reached (approximately 40-50 mg/kg/day for the rat), the higher NOAEL of 16.6 mg/kg/day is considered an appropriate point of departure.*

COMMENT 36: With regard to the discussion on selection of the principal study for the intermediate-duration oral MRL, the Reviewer questions "Why are Charles et al. 1996a; EPA 1996a,b not described here as candidate studies?"

RESPONSE: *The following statement was added:*

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

COMMENT 37: With regard to the discussion on selection of the principal study for the intermediate-duration oral MRL, the Reviewer comments "It is well established that 80+ nephrons have to be impaired for "renal functional impairment" to be detectable using standard urinalisis measures. Consistency of kidney as a target across studies and species, and evidence of other pathologies at the same NOAEL are all indicating that this may be a more appropriate and protective NOAEL. Yes, there was no intermediate

dose between 5 and 75 mg/kg in those studies, so one may argue that 16.6 mg/kg may be a more appropriate NOAEL, but an argument can be also made that the Marty et al study was of a shorter exposure… Selection of 5 mg/kg would seem as a more public health protective choice. Or a BMDL10 if the data are amenable to BMD modeling."

RESPONSE: *The kidney lesion incidence data were not amenable to BMD modeling because a dataset exhibiting a response only at the highest dose level would likely provide limited information regarding the shape of a dose-response curve. BMD analysis of kidney weight resulted in a higher point of departure ($BMDL_{1SD}$ of 34.12 mg/kg/day). As stated in Response to Comment 35, the 52-week study in rats (Charles et al. 1996a; EPA 1996a) identified a LOAEL of 75 mg/kg/day, which is higher than the LOAEL of 45.3 mg/kg/day identified by Marty et al. (2013). Based on the contention that adverse kidney effects occur when the saturation limit for kidney clearance of 2,4-D is reached (approximately 40-50 mg/kg/day for the rat), the higher NOAEL of 16.6 mg/kg/day is considered an appropriate point of departure.*

COMMENT 38: Regarding the subsection *Other Additional Studies or Pertinent Information that Lend Support to this MRL* in the MRL worksheet for the intermediate-duration oral MRL, the Reviewer states "No indication of why these studies were not chosen for NOAEL selection and/or BMD modeling… Especially since BMDL modeling was done for the 2 year data from these studies."

RESPONSE: *As stated in the Response to Comment 36, the discussion regarding selection of the principal study and critical effect was revised to include the results of the 1-year interim sacrifices in the Charles et al. (1996a) and EPA (1996a, 1996b) studies and why they were not selected as the critical studies.*

COMMENT 39: Regarding the uncertainty factors in the MRL worksheet for the chronic-duration oral MRL, the Reviewer comments "Additional uncertainty factor of 3 for database weaknesses may be warranted. Only 3 chronic studies are listed in the profile and the 1987 study did not provide adequate description of the renal lesions."

RESPONSE: *Available chronic-duration animal data suggest that increasing the 2,4-D oral exposure period from intermediate-duration to chronic-duration scenarios does not increase the sensitivity of the kidney to 2,4-D toxicity. ATSDR does not consider an additional uncertainty factor for database weaknesses to be necessary in this case.*

COMMENT 40: Regarding Section B.1.1, Literature Search, the Reviewer comments "The searches appear to have been limited to 2014 or later. This should be made clear in the summary. URLs for the databases/sources should be provided"

RESPONSE: *Text was updated to include date restrictions in the literature search:*

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

COMMENT 41: Regarding the description of the title and abstract screen in Section B.1.2, the Reviewer questions "By one person, or more? How were any inconsistencies resolved?"

RESPONSE: *The search was screened by one toxicologist and one chemist. The literature search was also submitted to ATSDR for review.*

COMMENT 42: Regarding the description of the title and abstract screen in Section B.1.2, Literature Screening, the Reviewer comments "Consider including appendix with listing of these studies and reasons for exclusion."

RESPONSE: *It is beyond the scope of the profile to include a listing of all studies identified in the literature search and the reasons for exclusion. ATSDR notes that the inclusion criteria used to identify relevant studies examining health effects are presented in Appendix B (Section B.1). Studies that were excluded did not meet these criteria.*

COMMENT 43: Regarding text in the full text screen summary in Section B.1.2, Literature Screening, compared to Figure B-1, January 2018 Literature Search Results and Screen for 2,4-D, the Reviewer notes "Literature tree mentioned 13 studies "excluded for criteria"… from 51 or 361? Please clarify"

RESPONSE: *The 13 studies were excluded from the combination of 361 previously cited studies and the 51 new studies identified in the update search.*

ATSDR Charge Questions and Responses

New intermediate oral MRL

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 44: The rationale for selecting kidney effects as the basis for derivation of the MRL is justifiable. This endpoint has been observed across numerous studies of 2,4D, in both males and females, and also has been reported in human case reports. Kidney plays important role in toxicokinetics of 2,4D and high dose effects on this organ are well explained, as noted by Marty et al (2013): "the transport of 2,4-D into the proximal tubule from plasma will saturate at a higher systemic concentrations in males than in females and thus allow for a greater delivered dose to proximal tubule cells." However, this reviewer was not clear as to why the longer duration studies (e.g., 12 month exposures) cited in the profile as (Charles et al. 1996a; EPA 1996a) have not been considered as a principal study. The reported NOAEL in those studies was 5 mg/kg/day, albeit the dose spacing has much to be desired. It was not clear whether those data were amenable for modeling or not. A justification for not selecting a lower NOAEL or deriving a BMDL from these other studies should be included if the Agency choses to retain Marty et al (2013) as the principal study.

RESPONSE: *As stated previously in the Responses to Comments 35 and 36, the MRL worksheet, in Appendix A, for the intermediate-duration oral MRL was revised to include the results of the 1-year interim sacrifices in the Charles et al. (1996a) and EPA (1996a, 1996b) studies and why they were not selected as the critical studies.*

New chronic oral MRL

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 45: Selection of (Charles et al. 1996a; EPA 1996b) for derivation of chronic oral MRL is justified. The choice of renal effects is good and the Agency performed benchmark dose modeling. This reviewer supports the choice of the model for deriving BMDL10 value. The only comment that this reviewer has is that consideration shall be given to adding a database uncertainty factor of 3. There are very few chronic studies of 2,4D and the study selected for deruivation of the MRL is the only suitable study with dose-response information and sufficient duration. Thus, the overall database is weak and this add to the uncertainty of the POD for derivation of the chronic oral MRL.

RESPONSE: *As stated previously, available chronic-duration animal data suggest that increasing the 2,4-D oral exposure period from intermediate-duration to chronic-duration scenarios does not increase the sensitivity of the kidney to 2,4-D toxicity. ATSDR does not consider an additional uncertainty factor for database weaknesses to be necessary in this case.*

Appendix A

QUESTION: Please address the MRL worksheets based upon the questions provided above about the MRLs.

COMMENT 46: Please see comments to the questions above.

RESPONSE: *All comments by the Reviewer regarding MRL worksheets have been addressed above.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

New intermediate oral MRL

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 1: I agree that the data from the study of Sturtz et al (2006) does not provide a good basis for an intermediate MRL. The principal reason is the one cited in the body of the profile (page 75) — the BMDL derived for depressed pup body weight from this study is inconsistent with other studies. EPA has speculated on the reason for the differences in dose-response relationships, but there is nothing known that would give this study greater weight than other studies that are well conducted and larger. Consequently, it does not represent well the body of information available on this endpoint and therefore would not be a good choice on which to base the MRL. That said, I'm not sure that this is sufficient reason not to include this study in Table 2-2. As a matter of transparency, and especially since this study was the basis of the intermediate MRL in a previous draft, it might be appropriate to include it in the table. The text can then help the reader see the inconsistency between the observations in this study and others, supporting the argument for its exclusion as a potential basis for an intermediate MRL. [I've seen this approach used in other profiles.]

RESPONSE: *ATSDR elected to summarize the results from Stürtz and coworkers in Section 2.17 of the toxicological profile along with statements regarding limitations of the studies and exclusion of the study results from potential consideration for MRL derivation. Because there is some uncertainty regarding the identification of the LOAEL, the study results were not included in LSE table (Table 2-2).*

New chronic oral MRL

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 2: I agree that the study and endpoint chosen for development of a chronic duration MRL are the most appropriate among the available options. The BMDL appears to have been calculated correctly and I concur with the choice of UFs. My only comment is that the source of data for his MRL should be cited as Charles et al. 1996b rather than 1996a. The latter is a study in rats, while the data for this MRL come from an oncogenicity study in mice presented in 1996b.

RESPONSE: *The Charles et al. (1996a) reference in Chapter 8 was supposed to be for the chronic-duration studies in rodents and the Charles et al. (1996b) reference was supposed to be the subchronic rat study. This error has been rectified in the revised Chapter 8.*

Appendix A

QUESTION: Please address the MRL worksheets based upon the questions provided above about the MRLs.

COMMENT 3: Repeating a comment above, for those focused on Appendix A it may seem odd that the study that was the basis of the intermediate MRL in a previous draft now seems to no longer exist. Consider including it in Table A-1 under developmental effects and explaining in text why it is not an appropriate basis for the MRL. Other than that, the Selection of the Critical Effect, Selection of the Principal Study, Summary of the Principal Study, and Selection of the Point of Departure for the MRL are fine. I agree with the use of a NOAEL/LOAEL approach giventhe less satisfactory (in my opinion) nature of the kidney weight endpoint and the results of the BMD modeling.

The MRL worksheet for the chronic oral MRL is straightforward and well written. The benchmark dose modeling appears to have been performed correctly using data from the mouse (Charles et al. 1996b !!).

RESPONSE: *ATSDR elected to summarize the results from Stürtz and coworkers in text of Appendix A along with statements regarding limitations of the studies. Because the results were not considered appropriate for MRL derivation, the study result was not included in Table A-1.*

Comments provided by Peer Reviewer #3

ATSDR Charge Questions and Responses

New intermediate oral MRL

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 1: The Reviewer did not provide comments.

RESPONSE: *No response is necessary.*

New chronic oral MRL

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 2: The Reviewer did not provide comments.

RESPONSE: *No response is necessary.*

Appendix A

QUESTION: Please address the MRL worksheets based upon the questions provided above about the MRLs.

COMMENT 3: I have reviewed the MRL worksheets in the subject document and here provide comments on the MRL derivations for the intermediate and chronic oral MRLs.

The intermediate oral MRL is based on a study by Marty et al. (2013).

1) Choice of NOAEL vs. BMDL: the journal article reporting this study does not include statistical measures or individual data for kidney weight. Did ATSDR attempt to contact the study author to obtain these data? Was the lack of these data th e reason for choosing the NOAEL rather than the BMDL? On p. A-12, this choice is presented as implicit and obvious: perhaps it was to the writer but not to the reader. Was the NOAEL chosen because the value was lower than that of the BMDL? In any case, the rationale for this choice should be made explicit.

RESPONSE: *BMD analysis was performed on the kidney weight data that were available to ATSDR in the form of an unpublished study (which was not cited in the toxicological profile because it is not available to the public). As stated in the MRL worksheet, the resulting* $BMDL_{1SD}$ *of 34.12 mg/kg/day (the benchmark response of 1 standard deviation from the control value) is higher than the NOAEL of 16.6 mg/kg/day. Therefore, the lower value (NOAEL of 16.6 mg/kg/day) was selected to be most public health protective. This rationale is stated in the MRL worksheet.*

2) Choice of uncertainty factors: Chemical-specific adjustment factors and data-derived extrapolation factors are preferable to default valued uncertainty factors. These are described in WHO-IPCS (2005) *Chemical-Specific Adjustment Factors for Interspecies Differences and Human Variability: Guidance Document for Use of Data in Dose/Concentration Assessment* and in USEPA (2014) *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation*. Was the choice of the default value of 10 for both animal-to-human extrapolation and human variability based on convention and ossified long-term practice or that insufficient data on renal effects in humans could be found to support different values. The basis for the choice should be stated.

RESPONSE: *ATSDR standard procedure is to apply default uncertainty factors of 10 for extrapolation from animals to humans and 10 for human variability in the absence of pharmacokinetic or pharmacodynamic data to suggest alternative uncertainty factors. ATSDR does develop chemical-specific uncertainty factors when data are available. The Agency did not consider the database on 2,4-D adequate to support derivation of chemical-specific uncertainty factors.*

COMMENT 4: The chronic oral MRL is based on a study by Charles et al. (1996).

1) The reference to Charles et al. (1996a) did not correspond to the study that provided the data. The three studies by these authors are:

Charles JM, Cunny HC, Wilson RD, et al. 1996a. Comparative subchronic studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in rats. Fundam Appl Toxicol 33(2):161-165.
Charles JM, Bond DM, Jeffries TK, et al. 1996b. Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxyacetic acid in rodents. Fundam Appl Toxicol 33(2):166-172.
Charles JM, Dalgard DW, Cunny HC, et al. 1996c. Comparative subchronic and chronic dietary toxicity studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in the dog. Fundam Appl Toxicol 29(1):78-85.

The second reference marked 1996b is actually the one used. All the citations in the chronic oral MRL derivation need to be checked for accuracy.

RESPONSE: *The studies of Charles et al. (1996a, 1996b) were inadvertently reversed in the reference chapter. The chronic study should have been Charles et al. (1996a). This has been corrected in Chapter 8 (References).*

2) The comments on the choice of uncertainty factors above is also applicable to the chronic oral MRL.

RESPONSE: *ATSDR's standard procedure is to apply default uncertainty factors of 10 for extrapolation from animals to humans and 10 for human variability in the absence of pharmacokinetic or pharmacodynamic data to suggest alternative uncertainty factors. As noted in the Response to Comment 3, the available database for 2,4-D did not support derivation of chemical-specific uncertainty factors.*