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

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Comments provided by Reviewer #1:

GENERAL COMMENTS

COMMENT: The report is a comprehensive evaluation and assessment of the epidemiologic, bioassay, and mechanistic literature to evaluate the link between exposure to 2,4-D and possible health effects in humans. The authors are to be congratulated on an excellent job of assembling, organizing and evaluating a very extensive and complex literature. Although I have several suggestions that I think will improve the clarity of the document, this does not detract from my overall conclusion that this is an excellent and well done effort.

RESPONSE: *No response is necessary.*

COMMENT: My one major concern about the document is a somewhat overly negative tone regarding the value of information from studies in humans. This gives the impression that information from epidemiologic studies is of limited value, which may lead the reader to conclude that such information may have been inappropriately dismissed or downgraded. A sentence on page 109 is presented as an example of this tendency. In referring to human studies it is stated that "As previously noted, being significantly associated does not imply causality". Does this mean that significant associations in humans can never imply causality? Would statistically significant associations from multiple studies of different designs, in different locations, and at different times still be insufficient? Though out the document there seems to be a need to point out "possible" general weaknesses in epidemiologic studies that are not as consistently raised for other disciplines. This gives the impression that human studies do not provide sufficiently reliable information to make much of a contribution in drawing conclusions about disease risks in humans. What is not found in the document are similar general statements about limitations in implying causality from experimental studies in animals. Could it not be said that any association observed between exposure and disease in animals does not imply causality for humans because of possible species differences and because exposure levels in many animal experiments are not reflective of what occurs for humans? The correct tone for the document should be that both experimental studies in animals and observational studies in humans make significant contributions to our understanding of health hazards. It is the balance of strengths and limitations of studies from various disciples that allows the overall research effort to be so successful in understanding human health issues.

RESPONSE: *The document did not intend to dismiss or downgrade results from epidemiological studies; rather, the document tried to put in context what associations mean or do not mean for readers who may not have a strong background in epidemiology and/or statistics. The statement about a significant association not necessarily implying causality was felt necessary for those who mistakenly assume that a statistically significantly increased risk means that exposure to the chemical caused the adverse health outcome. It does, however, indicate that exposure to the chemical plays a role in the health outcome, and as the number of studies showing statistically significant associations increase, so does the biological plausibility or the weight of evidence. This was added to the document.*

COMMENT: I wonder if it might be helpful to include a statement that the requirement to look at effects by route of exposure might somewhat diminish the utility of data from situations in humans where there is rarely a single route of exposure. The breakdown of exposure by inhalation, oral and dermal follows exposure routes, however, certainly works for experimental studies. Except for purposeful intoxication, however, most humans are exposed through multiple routes, usually by all three. Although the overall level of exposure may be known or estimated, the amounts associated with each route are

usually unknown and probably differ by study population. It seems cumbersome to repeat the human findings in each section, yet the findings should not be ignored either. This issue is raised in Section 3.2.1, but maybe needs visibility elsewhere also.

RESPONSE: *A sentence was added to the text in the introduction to Section 3.2.1 indicating that the reader needs to keep in mind that exposure usually occurs by multiple routes. The whole paragraph was also inserted at the beginning of Section 3.2.3, Dermal Exposure.*

COMMENT: Should there be a section that discusses production contaminants associated with 2,4-D, e.g., dioxins? It could include information on the presence or absence of particular types of contaminants, changes in possible concentrations over time, and how this might influence interpretation study findings?

RESPONSE: *The Reviewer's suggestion is beyond the scope of the document. In any case, contamination with dioxins does not seem to be a factor for 2,4-D as it is for 2,4,5-T.*

COMMENT: In multiple places in the document the idea is expressed that manufacturing workers have few exposures other than the active ingredient and many fewer other exposures than found in studies of applicators. I doubt this is correct. For industrial studies that have attempted to itemize the likely exposures beyond those of primary interest, the list is very long. Multiple exposures for individuals in the industrial setting are further increased because workers do not remain in one job over their entire work experience, even in a single plant. It is true that industrial studies are often written as if there are only a few exposures, but that does not make it so. The contention that manufacturing workers have few other exposures needs to be clearly supported by evidence. Documentation is necessary because the assumption of fewer other exposures leads directly to the conclusion that findings of exposure to 2,4-D from manufacturing studies are more relevant than from studies of applicators. This is despite the ability of some of the applicator studies to actually adjust for other possible exposures. The studies evaluating health effects from exposure to 2,4-D in manufacturing studies may not mention other simultaneous exposure, but such information might be obtained from monitoring studies, from the information on different exposures by industry available from NIOSH, and from production procedures and information regarding chemicals used in synthesis of 2,4-D. These sources should be evaluated and described in the document to support the contention that there are few other exposures in the manufacturing studies.

RESPONSE: *Statements indicating that workers involved in the manufacture of 2,4-D may have been exposed almost exclusively to 2,4-D were deleted from the document.*

COMMENT: The idea of a need for human studies of a pure exposure to 2,4-D is raised in several places in the document. I think this may have led to an inappropriate discounting of some studies. I doubt there are many, if any, humans exposed only to the active ingredient in 2,4-D. This is a desirable goal, but also it would seem important to develop an understanding about health hazards from the exposures that do occur in humans, even if not from just the active ingredient. It is appropriate to be concerned with exposures that may accompany use of 2,4-D because these co-exposures are potential confounders. On this point, however, there is no reason to restrict our concern about confounding to the substances that accompany the production or use of 2,4-D. A better approach would be to recognize that all studies of humans have multiple exposures (tobacco, alcohol, air pollution, multiple occupational exposures, household chemicals, etc.) that are not experimentally controlled and that it would be important to know the possible effects of 2,4-D in this mix. Although confounding should always be considered and evaluated, it is important to remember that for it to actually occur the putative confounder must be tightly

correlated with the agent of interest and it must also cause the outcome of concern. These two absolute requirements occur relatively rarely.

RESPONSE: *Text in the profile where the Reviewer made specific comments regarding human exposure to 2,4-D were revised following the Reviewer's suggestions listed below.*

*The page numbers given here are the numbers of the pages in the entire document, starting with the title page as Page 1.

CHAPTER 1. PUBLIC HEALTH STATEMENT

COMMENT: Page 22, line 15: I think you need a qualifier here because duration and amount of exposure can be overlapping concepts. Amount is clearly important, but duration divorced from amount may or may not be.

RESPONSE: *The comment refers to standardized text at the beginning of Chapter 1: "If you are exposed to 2,4-D, many factors determine whether you'll be harmed. These include how much you are exposed to (dose), how long you are exposed (duration), and how you are exposed (route of exposure). You must also consider the other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health." The Reviewer's suggestion will be considered for future profiles.*

COMMENT: Page 22, line 32: The two half life sentences seem inconsistent. Previous sentence says ½ life is 19 hours in air and that it breaks down quickly in the soil, but the ½ life is 6 days or longer. Needs some clarification.

RESPONSE: *The wording has been changed to indicate that it is not persistent in soil. Comparing half-lives in two mediums (air and soil) is not relevant since degradation occurs by two different processes. What is considered long in one may be short in another by nature of the degradation mechanisms. A soil half-life of 6 days is considered rapid degradation for a pesticide. As a comparison, the half-life of long-lived pesticides like DDT is years.*

COMMENT: Page 23, line 15: Probably need some clarification as to what "high" is here. I think you mean exposures from food and water are likely to be less than from application. A citation with evidence would be nice.

RESPONSE: *ATSDR has added references from the U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) along with text describing infrequent detection in food items and low levels of detection in drinking water.*

COMMENT: Page 23, line 22: Is it correct that only a small amount enters through the skin or lungs, or is it the absorption rate is lower for the skin and lungs than through the gastrointestinal tract? The actual amount entering depends upon the amount brought into contact with the various tissues. My understanding is that the amount that comes into contact with the skin and lungs is significantly greater than through the digestive tract for most people.

RESPONSE: *In this section, the text is only indicating that 2,4-D can enter the body, not which route is the main route of exposure for the general population. Reference to the lungs was deleted since there are no quantitative data for this specific route.*

COMMENT: Page 24, line 29: Why are papers that do not show strong links to "other cancers" cited, but earlier in the paragraph papers with some associations are not?

Need to add the citation for the IARC evaluation here because it provides information on "other cancers."

RESPONSE: *The references at the beginning of the section are citations of case reports. Burns and Swaen (2012), Garabrant and Philbert (2002), and von Stackelberg (2013) are reviews that include non-Hodgkin's lymphoma (NHL) and other cancers. They were not placed in the text as support for negative associations between2,4-D and cancer. These reviews are cited instead of the enormous number of individual studies showing positive and negative associations. References in Chapter 1 are deleted from the document that is published for public comments and from the final document. The text was revised so that it does not appear that Burns and Swaen (2012), Garabrant and Philbert (2002), and von Stackelberg (2013) are in support only for the sentence on other types of cancer. Reference to IARC was added.*

COMMENT: Page 24, line 30: The studies by Hayes et al. on dogs did find an association between 2,4-D and lymphoma. If you have doubts about it, you can enter them here also. Also I thought the recent IARC review concluded there were positive animal bioassay results (you should check).

RESPONSE: *The results of Hayes et al. (1991) in household dogs are controversial and have been disputed, so ATSDR preferred not to mention them in Chapter 1, but they are mentioned in Section 3.2.3.7, Cancer. IARC published the conclusions of the Working Group in an article in Lancet Oncol (Loomis et al. 2015), which includes a summary of the human and animal cancer data. This report was added to the profile. The full assessment, Monograph 113, was recently published online and was also added to the profile. IARC (2016) classified 2,4-D as "possibly carcinogenic to humans" based on "inadequate evidence" in humans and "limited evidence" in experimental animals.*

COMMENT: Page 25, line 2: Wording seems a little unbalanced here. EPA classification is based on "not enough information" but the IARC review a decade later is not explained. As worded you might conclude that there should also not have been enough information for IARC evaluation either.

RESPONSE: *The text was revised to state that, according to EPA, not classifiable means: "…without adequate data to either support or refute human carcinogenicity." In addition, text was added to explain the basis for IARC's classification, inadequate evidence in humans and limited evidence in animals.*

COMMENT: Page 25, line 17: Are there any "drawbacks" to the studies that show no effects? There is a tendency to accept negative studies as gospel, but positive ones as full of weaknesses. The opposite is often true because it is easier for limitations to cause false negatives than false positives.

RESPONSE: *All of the studies cited in this paragraph have limitations inherent to epidemiological studies, but it would not be appropriate to discuss each study's strengths and limitations in Chapter 1. Instead, a sentence was added stating that limitations may have influenced the results into finding positive or negative links, so no firm conclusions can be drawn.*

COMMENT: Page 25, line 20: Indicate what the limitations were and how that should temper your conclusions. The current wording might imply the study results should be dismissed. Is that what is intended? In general, I do not like the phrase "should be taken with caution" because this could probably be applied to nearly every study and the reader does not know what to do with it. Also it is not clear how the authors have tempered their interpretation.

RESPONSE: *The text was revised as indicted above.*

COMMENT: Page 26, line 2: Not clear on what this advice is based? How would the doctor "find" a person has been exposed to significant amounts? Could be by blood or urine analysis immediately but only immediately after exposure, but this would be highly unusual. More likely the person would tell the doctor they were exposed. This seems to put the responsibility on the doctor to define exposure and is unlikely to bring any new information to the issue in most situations.

RESPONSE: *The comment refers to standardized text at the beginning of the section How Can Families Reduce the Risk of Exposure to 2,4-D? This text appears in all profiles. ATSDR will consider the Reviewer's suggestion for future profiles.*

COMMENT: Page 26, line 8: Should add a few citations on exposure studies that have demonstrated that protective equipment reduces exposure. Could start with: Thomas KW et al. Assessment of a pesticide exposure intensity algorithm in the agricultural health study. J Expos Sci Environ Epidemiol 2010; 20(6):559-569.

RESPONSE: *ATSDR has added the requested citation to this section.*

COMMENT: Page 26, line 11: Should comment on some studies of dogs that demonstrate 2,4-D is found in the urine of dogs allowed on lawns treated with the herbicide and this is relevant to human exposure in this situation.

RESPONSE: *ATSDR has added a study that shows that 2,4-D is in the urine of dogs that may have been exposed to the herbicide from their owner's treated lawn or from other lawns that the dogs may have visited.*

COMMENT: Page 26, line 19: Probably should provide an indication of the ½ life here.

RESPONSE: *A half-life was added to this section.*

COMMENT: Page 27, line 9: I think a few examples of differing "not-to-exceed" levels might be helpful.

RESPONSE: *The comment refers to standardized text at the beginning of the section regarding federal guidelines and recommendations. This text appears in all profiles. ATSDR will consider the Reviewer's suggestions for future profiles.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

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

COMMENT: Page 29, line 15: Given the great increase in the use of glyphosate, more recent information and references are needed.

RESPONSE: *ATSDR has added quantitative data for 2,4-D usage in the United States from 2013, the last year data were available from the USGS.*

COMMENT: Page 30, line 1: Any references with measurements in food?

RESPONSE: *ATSDR has added the FDA (2005) reference for the 2004–2005 Total Diet Studies discussed in Section 6.4.4.*

COMMENT: Page 30, line 7: Earlier discussion about exposure implied that ingestion might be the major route. I think this was because the early discussion was focusing on rate of absorption rather than amount of exposure, but this is not entirely clear.

RESPONSE: *ATSDR has altered the wording to read that 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).*

COMMENT: Page 30, line 12: Probably correct, but probably should mention the exposure possibility from play in areas treated with 2,4-D, i.e., yards. Here the monitoring studies in dogs are important.

RESPONSE: *ATSDR has referenced the study by Nishioka et al. (2001), which indicated that track-in practices from homeowners and their pets were a potential source of 2,4-D in the home.*

2.2 SUMMARY OF HEALTH EFFECTS

COMMENT: Page 30, line 23: If you provide citations for studies of manufacturers, you also need to provide them for studies of farmers and other applicators.

RESPONSE: *The citations are reviews that cover manufacturers as well as farmers and other applicators. This was preferred over listing an enormous number of individual references. References in Section 2.2 are deleted before the document is finalized in order for the section to read more like an executive summary. Information summarized in Chapter 3, however, is from original references, which are retained in the final document.*

COMMENT: Page 30, line 34: Need to be clear what "interpreted with caution" means. Is it not to be believed? Worried but need more evidence? See earlier comments on this point.

RESPONSE: *The statement about caution was replaced with text indicating that the estimated lethal doses represent the combined action of 2,4-D and other substances present in the commercial products.*

COMMENT: Page 31, line 9: Papers from the Agricultural Health Study that would be relevant here include:

> DeRoos AC, Cooper GS, Alavanja MC, Sandler DP. Rheumatoid arthritis among women in the Agricultural Health Study: Risk associated with farming activities and exposures. Amm Epidemiol 2005; 15:762-770.
>
> Hoppin CR, Umbach DM, London SJ, Lynch CF, Alavanja MCR, Sandler DP. Pesticides associated with wheeze among commercial applicators in the Agricultural Health Studies. Am J Epidemiol 2006; 163:1129-1137.
>
> Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MCR, Sandler DP, Hoppin JA. Chronic bronchitis among non-smoking farm women in the Agricultural Health Study. J Occup Environ Med 2007; 49:574-583.
>
> Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MCR, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. Am J Respir Crit Care Med 2008; 177:11-18.

RESPONSE: *Information from the four studies was added to the appropriate sections in the profile.*

COMMENT: Page 31, line 12: I do not think the phrase "great majority did not" accurately portrays the literature.

RESPONSE: *"Great majority" was changed with "some".*

COMMENT: Page 31, line 15: This sentence seems overly negative. ALL occupations have exposure to "multiple chemicals" yet this has not prevented uncovering many clear links between disease and occupational exposures. Some studies actually deal directly with this possible confounding. Other exposures are irrelevant unless they also cause the disease of interest. This alone eliminates most exposures as confounders. Which exposures are you postulating cause the diseases observed and, therefore, might be confounders?

RESPONSE: *As previously indicated, the statement about a significant association is not necessarily implying that causality was felt necessary for those who mistakenly assume that a statistically significantly increased risk means that exposure to the chemical caused the adverse health outcome. It does, however, indicate that exposure to the chemical plays a role in the health outcome, and as the number of studies showing statistically significant associations increases, so does the biological plausibility and the weight of evidence. This was added to the document.*

COMMENT: Page 31, line 18: These statements are correct, but are relevant only if they apply to the studies being considered here. Which studies that show an effect had "too few cases reported for a meaningful and which ones did not include frequency of use? Should not just imply that all studies have these problems, if they do not. And interpretation." As worded this sentence implies that all positive

findings suffer from these limitations. Is this the case? Also can these two limitations affect non-positive studies?

RESPONSE: *The text was revised to indicate that limitations apply to studies that reported positive associations as well as to studies that reported negative associations.*

COMMENT: Page 31, line 24: Must cite the recent IARC review here.

RESPONSE: *The IARC Monograph (Volume 113) that summarizes all of the information that IARC reviewed in support of the recent classification was recently published online. Relevant sections of the profile were revised accordingly.*

COMMENT: Page 31, line 26: Zahm et al. (1990) study also showed an association

RESPONSE: *Zahm et al. (1990) found that among those that applied or mixed 2,4-D for 21 or more days per year, the odds ratio (OR) increased 3-fold (OR 3.3, 95% confidence interval [CI] 0.5–22.1) based on only three cases and four controls, which is reflected in the wide interval. There was no significant association with years of 2,4-D used on farm or based on first year of 2,4-D use.*

COMMENT: Page 31, line 26: Should cite the studies that have definite information on 2,4-D use that do not show and association. As worded it implies that the number that do not show an association is much larger than those that do. This is not correct.

RESPONSE: *The text was revised to indicate that some studies showed a positive association while other did not.*

COMMENT: Page 31, line 29: There is a more recent Burns paper on this. It did show some excesses.

RESPONSE: *The text refers to statistically significant associations. In Burns et al. (2011), the Standardized Incidence Ratio was 1.36 (95% CI 0.64–2.29).*

COMMENT: Page 31, line 30: Were numbers too small for evaluation of specific pesticides and childhood cancer?

RESPONSE: *Yes, there were very few cases, which was noted by Flowers et al. (2004): "The small number of cases and limited statistical power may have prevented us from detecting an association between frequency of use and childhood cancer risk." This was mentioned above as one of the limitations encountered by epidemiological studies in general.*

COMMENT: Page 32, line 10: In the more recent follow-up of the Dow cohort by Burns (Burns C et al. Cancer incidence of 2,4-D production workers. Int J Environ Res Public Health 2011; 8:3579-3590) NHL was slightly increased and relative risks were greatest in the upper duration and cumulative exposure categories (although neither was statistically significant). This should not be classified as not suggestive patterns. The Bond et al. (1988) study is just an earlier version of the Burns publication, so I should not be counted as a separate study.

RESPONSE: *Burns et al. (2011) did evaluate risk of various cancers, but not specifically mortality. However, the increased risk of NHL with duration and cumulative exposure reported by Burns et al. (2011) was added in the NHL section in Chapter 3.*

COMMENT: Page 32, line 13: Need to provide documentation that this assumption of "less confounding" is correct. I doubt that it is. Manufacture and formulation operations typically include use of a number of feedstock chemicals and end products. Often the number of chemical exposures in manufacture and formulation operations for pesticides is as great, and typically greater than for those workers in the application operations. Studies that I have done in the industrial setting have hundreds of possible exposures.

It is important to remember that "confounding" is defined not by concurrent exposure, but by a concurrent exposure that "causes" the disease of interest. This considerably narrows the number of possible confounders.

RESPONSE: *As previously mentioned, statements indicating that workers involved in the manufacture of 2,4-D may have been exposed almost exclusively to 2,4-D were deleted from the document.*

COMMENT: Page 32, line 16: I think the human route of exposure is most likely inhalation or skin. If this is correct, do the different routes of exposure for animals and humans influence interpretation of possible human risk from animal studies?

RESPONSE: *Yes. It is always better to have studies in animals by the route of exposure that is most relevant to human exposures. Regrettably, only one inhalation study in animals was available for review, and this is a data need. There are no established methodology for derivation of dermal Minimal Risk Levels (MRLs), so even if there were adequate dermal animal studies, dermal MRL would have not been derived. Perhaps a data need should be to develop physiologically based pharmacokinetic (PBPK) models that can be used to perform animal-to-human and route-to-route extrapolations.*

COMMENT: Page 33, line 5: Although these studies observed different LOAELs, is not possible to at least set a range of possibility, or an upper limit at which effects are likely to be seen? Since they all showed some effect, seems the interpretation could be based on that.

RESPONSE: *It unclear what the interpretation could be. It is clear that exposure to 2,4-D can induce adverse renal effects in animals. Table 2-1 shows LOAELs for renal effects ranging from 5 to 300 mg/kg/day in rats and from 15 to 430 mg/kg/day in mice. The question is why the range is so large. As the text notes, it could be due to the manner the dietary amounts (ppm in the diet) were converted to doses (mg/kg/day) in the different studies. Still, the LOAEL of 2.5 mg/kg/day for developmental effects in rats from Stürtz et al. (2010) is lower and was used for MRL derivation.*

COMMENT: Page 33, line 21: Need to explain why these data are "rather limited"

RESPONSE: *Text was revised to state that the information available is insufficient to draw conclusions regarding 2,4-D and the immune system.*

COMMENT: Page 34, line 11: It is not clear that the outcomes for "Joshi et al" are the same as cited from the studies in the previous sentence. The previous sentence does not mention Sertoli or Leydig cells or reduced sperm count.

RESPONSE: *It was specified that the discrepancy refers to sperm parameters.*

COMMENT: Page 35, line 2: Should also include what IARC concluded about 2,4-D mechanistic actions. Would be informative to indicate how IARC arrived at the "possible" classification, i.e., inadequate in humans, possible in animals, and strong mechanistic evidence.

RESPONSE: *The text was revised to include IARC's recently published full assessment of 2,4-D's carcinogenicity (IARC 2016), the basis for the classification, and the potential mechanisms involved.*

2.3 MINIMAL RISK LEVELS (MRLs)

Inhalation MRLs

COMMENT: Page 36, line 3: Indicate why the database is "insufficient."

RESPONSE: *It seems unnecessary to explain that a single study constitutes an insufficient database.*

Oral MRLs

COMMENT: Page 36, line 20: The concern that other chemicals in the commercial formulations may contribute to the toxicity is important if the goal is to know with absolute assurance which chemical is involved. It is less important if the issue is to prevent human ill health. Are there any human populations that would be exposed to only the pure active ingredient 2,4-D? Certainly all applicators and environmental exposures would be exposed to the formulation. And workers in most manufacturing facilities also have multiple exposures. It seems to me some comments are necessary to indicate that this is the "exposure" that most humans get.

In addition, there are different formulations, but the common exposure across these is the active ingredient.

RESPONSE: *The text was revised incorporating the points that the Reviewer makes, that most environmental exposures are not to pure 2,4-D, but to a combination of chemicals.*

COMMENT: Page 37, line 11: Perhaps just provide the number of studies that saw effects below and above 100 mg/kg. This would be more informative that the Judgment that there are "very few exceptions". Could also cite those that did not find effects below 100.

RESPONSE: *For clarity, the sentence: "With very few exceptions, doses ≤100 mg 2,4-D/kg did not induce toxic effects in acute-duration oral studies in animals" was deleted. The purpose of the discussion in this section is identifying the lowest LOAELs, so citing studies that did not find effects below 100 mg/kg is not very useful.*

COMMENT: Page 38, line 10: Would information on dogs be useful as an indicator for more sensitive humans, i.e., the young, the old, or the ill? Also is there natural genetic variation on sensitivity in humans for which dogs might provide some useful information?

RESPONSE: *There is no information about genetic variations in humans regarding the renal OAT1 activity carrier involved in excretion of 2,4-D, so it is mentioned in Section 3.12.2 as a data need (Comparative Toxicokinetics).*

COMMENT: Page 41, line 18: Seems a little odd to me that histologic alterations of the kidney might not be considered "adverse" but body weight changes would.

RESPONSE: *Histological alterations in the kidneys are considered adverse and are plotted as such in Table 3-2. However, the maternal dose level of 2.5 mg 2,4-D/kg/day for decreased body weight gain rat pups reported by Stürtz et al. (2010) is still lower than the lowest LOAEL for renal effects (5 mg/kg/day), so it was selected as basis for the MRL.*

COMMENT: Page 42, line 29: How do humans and mice/rats compare regarding 2,4-D elimination? If information on dogs are discounted because of differences with humans, you should present the data on rodents and humans here to show they are similar.

RESPONSE: *This is discussed in Section 3.5.1. Pharmacokinetic Mechanisms. Plasma half-lives were 0.8–2.1 hours in rats and mice, 92–106 hours in dogs, and 12 hours in humans. These values were estimated for an ingested dose of 5 mg/kg (Timchalk 2004). Based on this, humans are less susceptible than dogs. Reference was made to Section 3.5.1 on page 42, line 29.*

CHAPTER 3: HEALTH EFFECTS

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

COMMENT: Page 45, line 23: I wonder if there is a need for clarification of what "a day" means? Does this mean any exposure, no matter how brief, in a day, a full 8-hour work day, or a 24-hour environmental exposure?

RESPONSE: *The comment refers to standardized text that appears at the start of Section 3.2, DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE. Additional clarification seems unnecessary, 14 days or less is self-explanatory. However, the type of effect resulting from the exposure would dictate whether or not an MRL is derived.*

3.2.1 Inhalation Exposure

COMMENT: Page 46, line 27: For humans you might consider adding a section about the impact of protective gloves, clothing and boots on the relative contribution from dermal versus inhalation exposures. As dermal exposures are reduced the relative contribution of inhalation increases. This could be important because I think equipment to protect from inhalation is rarely used, at least in application.

RESPONSE: *Information relevant to this comment is provided in Section 6.5, GENERAL POPULATION AND OCCUPATIONAL EXPOSURE.*

COMMENT: Page 46, line 28: Perhaps move these comments to "bolded" section above. Include some discussion on the usefulness and limitations of the route

RESPONSE: *The bolded section is standardized text that appears in all profiles; text from other section cannot be moved into a standardized text section. Text was added to the end the first paragraph of Section 3.2.1 as a result of comments from another reviewer stating that 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.*

3.2.2 Oral Exposure

COMMENT: Page 50, line 11: The comment refers to the following text: "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." Could look at the literature for these other exposures to see if there is evidence that they cause the same effects as found in the studies of 2,4-D. For most I suspect this is not going to be the case. This would provide a clearer indication regarding possible confounding and if it is a real cause for concern.

RESPONSE: *It is beyond the scope of the profile to provide information on the toxicity of chemicals present in commercial formulations of pesticides.*

3.2.2.2 Systemic Effects

Respiratory effects

COMMENT: Page 52, line 3: Atopic asthma has been reported in farmers using 2,4-D (Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MCR, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. Am J Respir Crit Care Med 2008; 177:11-18.)

RESPONSE: *Information from the study was added to the profile.*

COMMENT: Page 52, lone 7: Sentence says there is "one" positive report, but the next sentence appears to cite 4 studies.

RESPONSE: *The text was revised, "This was the case…" was replaced with "No significant effects were reported in …"*

Cardiovasular effects

COMMENT: Page 53, line 4: Does this mean similar to the paragraph above, i.e., no effects?

RESPONSE: *Yes, but for clarity the text was revised so the sentence starts: Similar* ***negative*** *results…*

COMMENT: Page 53, line 8: Why are only animal results considered for the conclusion?

RESPONSE: *The sentence was revised as follows: "Based on the information available, it does not appear that the cardiovascular system is a sensitive target for 2,4-D."*

Gastrointestinal effects

COMMENT: Page 54, line 2: Probably need to make clear that "relatively high doses" does not include amounts consumed by humans that caused death. Are the effects from ingestion cases in humans not useful? Probably need to indicate why they are discounted.

RESPONSE: *The comment refers to the sentence: "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." The sentence is accurate, and in fact, is consistent with the limited human data from cases showing adverse effects following bolus ingestion of products containing 2,4-D. Human data are not discounted.*

Hematological effects

COMMENT: Page 54, line 7: Seems odd to start the description with "No meaningful data were available" then to describe some apparently "meaningful" data.

RESPONSE: *The text was revised by changing* ***No meaningful data****… with* ***The only data…***

Hepatic effects

COMMENT: Page 56; line 3: How about humans?

RESPONSE: *The comment refers to the following sentence: "In general, results suggest species differences in sensitivity, with dogs being more sensitive than rodents." No data were located regarding hepatic effects in humans following long-term oral exposure, so no comparisons with animals can be made.*

Renal effects

COMMENT: Page 58, line 11: Were any of the kidney effects in animals similar to those observed from fatal cases in humans?

RESPONSE: *It seems that one would not expect kidney effects seen in fatal human cases in which the subjects ingested a commercial herbicide formulation containing 2,4-D as a bolus to be similar to those seen in animals following long-term dietary exposure to 2,4-D. High-dose acute studies in animals that assessed lethality did not provide information regarding kidney effects, most likely because death was usually attributed to adverse neurological effects. As indicated in the profile, renal congestion, but no degenerative changes, was reported in one fatal case (Nielsen et al. 1965) and mildly active chronic*

pyelonephritis, moderate arteriolar sclerosis, congestion of the capillaries of the medulla, and dilated collecting tubules were reported in another case (Dudley and Thapar 1972). Acute kidney failure reported in a third fatal case (Keller et al. 1994); no information was given as to how the kidney failure was diagnosed.

Body Weight effects

COMMENT: Page 60, line 23: Although exposure is probably mainly occurs through the skin and lungs, there has been a study in humans on 2,4-D and BMI, which found no association. (Laverda N, Goldsmith DF, Alavanja MCR, Hunting K: Pesticide Exposures and Body Mass Index (BMI) of Pesticide Applicators from the Agricultural Health Study. J Toxicol Environ Health Part A: 2015; 78:1-20 (DOI:10.1080/15287394.2015.107/4844).

RESPONSE: *The study was added to Section 3.2.3.*

3.2.3 Dermal Exposure

3.2.3.1 Death

COMMENT: Page 69, line 30: In other discussions of human studies, multiple exposures for occupational groups is listed as a limitation and a common exposure to 2,4-D is not listed as a strength. All occupational studies have these two conditions.

RESPONSE: *The issue of exposure to 2,4-D being the common factor between studies has been addressed throughout the document. For example, it is now mentioned in Chapter 2 and at the beginning of Section 3.1, Inhalation Exposure.*

Endocrine effects

COMMENT: Page 72, line 31: There are a couple of papers from the Agricultural Health Study that provide information on 2,4-D and thyroid problems:

> Hypothyroidism has been associated with 2,4-D exposure in applicators (Goldner WS, Sandler DP, Yu F, Shostrom V, Hoppin JA, Kamel F, LeVan TD. Hypothyroidism and pesticide use among male private pesticide applicators in the Agricultural Health Study. JOEM 2013; 55:1171-1178.)
>
> Thyroid disease among women was not associated with 2,4-D in AHS (Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, Levan TD. Pesticide use and thyroid disease among women in the Agricultural Health Study. Am J Epidemiol 2010; 171:455-464.

RESPONSE: *Information from these studies was added to the profile.*

Ocular effects

COMMENT: Page 73, line 16: No association was found between 2,4-D and retinal degeneration and other eye disorders among wives with possible exposure to 2,4-D in the Agricultural Health Study. Am J Epidemiol 2005; 161:1020-1029.

RESPONSE: *Information from Kirrane et al. (2005) was added to the profile.*

COMMENT: Page 73, line 27: Study in the AHS found no statistically significant association between birth weight and maternal exposure to 2,4-D. (Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, Hoppin JA. Maternal pesticide use and birth weight in the Agricultural Health Study. J Agromedicine 2010; 15:127-136.)

RESPONSE: *Information from Sathynarayana et al. (2010) was added to the profile.*

3.2.3.3 Immunological and Lymphoreticular Effects

COMMENT: Page 74, line 4: See papers below that might be relevant here or elsewhere.

1) Hofmann JN, Hoppin JA, Lynch CF, Blair A, Alavanja MC, Beane Freeman L. Farm characteristics, allergy symptoms, and risk of non-Hodgkin lymphoid neoplasms in the Agricultural Health Study. Cancer Epid Biomarkers Prev 2015; 24:587-594.

2) Hou L, Andreotti G, BaccarelliAA, Savage S, Hoppin JA, Sandler DP, Barker J, Zhu AA, Hoxha M, Dioni L, Zhang X, Koutros S, Beane Freeman LE, Alavanja MC. Lifetime pesticide use and telomere shortening among male pesticide applicators in the Agricultural Health Study. Environ Health Perspect 22013; 121:919-924.3)

3) Andreotti G, Hoppin JA, Hou L, Koutros S, Gadalla SM, Savage SA, Lubin J, Blair A, Hoxha M, Baccarelli A, Sandler D, Alavanja M, Beane Freeman LE. Pesticide use and relative leukocyte telomere length in the Agricultural Health Study. PLOS One 2015;woi:0.137/journal.pone.0133382.

4) Figgs LW, Holland NT, Rothman N, Zahm SH, Tarone RE, Hill R, Vogt RF,Smith MT, Boysen CD, Holmes FF, VanDyck, Blair A. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. Cancer Cause Control 2000; 11:373-380.

RESPONSE: *Data from Hou et al. (2013), Andreotti et al. (2015), and Figgs et al. (2000) are presented in Section 3.3, GENOTOXICITY. There is no specific information regarding 2,4-D in Hofmann et al. (2015).*

3.2.3.5 Reproductive Effects

COMMENT: Page 75, line 29: Also: Arbuckle TE, Savitz DA, Mery LS, Curtis KM. Exposure to phenoxy herbicides and the risk of spontaneous abortion. Epidemiology 10:752–760 (1999).

RESPONSE: *Data from the study were added to the profile.*

3.2.3.7 Cancer

NHL

COMMENT: Page 77, line 34: The finding of significantly elevated OR among those not using protective equipment suggests that they actually have the highest exposure in this study. This should be mentioned

RESPONSE: *The Reviewer's suggestion was added to the text.*

COMMENT: Page 78: line 3: It is correct that Cantor et al. (1992) did not find a statistically significant excess, but there was a small excess. However, there was not much of an exposure response (Cantor KP et al. Letter to the Editor Correspondence. Pesticides and Other Agricultural Risk Factors for Non-Hodgkin's Lymphoma among Men in Iowa and Minnesota. CANCER RESEARCH 53. 2421. May 15. 1993]

RESPONSE: *It unclear what the suggestion is. The Cantor et al. (1993) letter is consistent with the results presented in the full paper.*

COMMENT: Page 78: line 3: With the significant excess among those not using protective equipment in this study, I do not think it should be classified as having "no significant association."

RESPONSE: *The Miligi et al. (2003) reference was deleted since the population studied is the same as that reported by Miligi et al. (2006), mentioned a few lines above in the text of the profile.*

COMMENT: Page 78: line 4: The Zahm et al. (1990) in Nebraska found an association and exposure-response pattern between 2,4-D and NHL.

RESPONSE: *As mentioned earlier, Zahm et al. (1990) found that among those that applied or mixed 2,4-D for 21 or more days per year, the OR increased 3-fold (OR 3.3, 95% CI 0.5–22.1) based on only three cases and four controls, which is reflected in the wide interval. There was no significant association with years of 2,4-D used on farm or based on first year of 2,4-D use.*

COMMENT: Page 78: line 8: The study by Burns et al. (2011)) has some cancer excesses worth discussing (other respiratory, prostate and NHL). There are significant trends between NHL and duration and cumulative exposure to 2,4-D.

RESPONSE: *The prostate and respiratory findings from Burns et al. (2011) are mentioned in the appropriate sections discussed below the NHL. The increased ORs for NHL with duration and cumulative exposure in the Burns et al. (2011) were added to the text.*

Hodgkin's disease

COMMENT: Page 78: line 17: I did not check the non-North American studies to see if 2,4-D use is sufficient to in these studies to provide useful evidence on 2,4-D exposure. My recollection is that 2,4-D was very rarely used in Europe (although other phenoxy acids other than 2,4,5-T were).

RESPONSE: *Apparently there was some use. However, as the text indicates, in the Kogevinas et al. (1995) study, 2,4-D was not assessed individually, but combined with 2,4-DP and 2,4-DB.*

COMMENT: Page 78: line 17: Should note that this analysis was based on only 5 cases.

RESPONSE: *On line 14, it is noted that the analysis was based on only five cases.*

Leukemia

COMMENT: Page 79, line 7: It would be better to list the "limitations" and the effects they might have on the relative risks. Not sure what the phrase "should be taken with caution." Means. Does it mean discout entirely, only consider in some circumstances, or something else?

RESPONSE: *The statement about the limitations was deleted as a result of comments provided by another reviewer.*

Prostate Cancer

COMMENT: Page 81, line 11: This is a prospective cohort study, not a nested case-control.

RESPONSE: *The comment refers to the Agricultural Health Study. The text was revised.*

COMMENT: Page 81, line 22: I wonder if you might want to be a little clearer about your conclusion here. There are usually some inconsistency among studies. It is okay to say the inconsistencies are so great that you do not want to make any statement about the possibility of hazard. As now stated, however, a meaningful conclusion has been drawn, i.e., that despite several positive studies the combined evidence does not even suggest an association.

RESPONSE: *The text was revised with the statement suggested by the Reviewer.*

Other Cancers

COMMENT: Page 81, line 30: Double check to makes sure this is what the authors said prevented access to job histories. Other Dow studies have reported on detailed job histories.

RESPONSE: *Burns et al. (2011) states the following on page 3585 of the study: "Because of identification of the incident cases was preclude due to the confidentiality agreement with the MDCH, we were unable to scrutinize their job histories."*

COMMENT: Page 82, line 1: Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MCR. Pesticide use and colorectal cancer risk in the Agricultural Health Study. Int J Cancer 2007; 121:339-346

RESPONSE: *Lee et al. (2007) is mentioned under gastrointestinal cancers.*

RESPONSE: *A sentence regarding the findings of Reynolds et al. (1994) was added to the text.*

3.3 GENOTOXICITY

In vivo Exposure Studies

COMMENT: Table 3-4, Page 84 (Genotoxicity of 2,4-D In Vivo): Holland et al. (2002) also found in increase in the replication index for lymphocytes. They also found it was stronger for commercial 2,4-D than for the chemically pure ingredient.

RESPONSE: *It is hard to draw conclusions from the results regarding replicative index (RI) in the Holland et al. (2002) study. In Table 1 of the study, the mean RI in lymphocytes from five unexposed donors incubated with 0.005 mM pure 2,4-D was 1.59 compared with 1.69 incubated with a commercial mixture. The results reversed when the concentration of 2,4-D was 0.3 mM, 1.29 vs. 1.24. Figure 2 in the study shows the variability between the donors.*

COMMENT: Page 88; line 2: Andeotti et al paper has been published (PLOS One (2015)). Also there is a paper by Hou on the AHS that uses buccal cells to evaluate telomere length.

RESPONSE: *Both the Hou and the Andreotti studies were added to the text and Table 3-4.*

COMMENT: Page 88, line 12: Should point out that the proliferation index increases in the exposed group after first exposure and was also greater among the exposed than among a control group of non-applicators.

RESPONSE: *The statement provided by the Reviewer was added to the text.*

COMMENT: Page 88, line 14: There was an experimental paper by Holland (2002) in Mutation Research that showed a proliferation effect.

RESPONSE: *As indicated above, it is hard draw conclusions from the results regarding replicative index (RI) in the Holland et al. (20o2 study. In Table 1 of the study, the mean RI in lymphocytes from five unexposed donors incubated with 0.005 mM pure 2,4-D was 1.59 compared with 1.69 incubated with a commercial mixture. The results reversed when the concentration of 2,4-D was 0.3 mM, 1.29 vs. 1.24. Figure 2 in the study shows the variability between the donors.*

In vitro Studies

COMMENT: Page 90, line 8: I assume that the conclusion for "Genotoxicity" is that the data are insufficient to make any statement about 2,4-D. Given the number of associations in human, animal and

in vitro studies, I think this conclusion needs to be explained more fully. Be clear about why all or most of the positives must be discounted, or dismissed. This is necessary because it appears to contrary to the IARC conclusion.

RESPONSE: *The conclusion was revised to indicate that the positive results support a biological plausibility and cannot be discounted. It should be noted that IARC (2016) concluded that the evidence that 2,4-D is genotoxic is weak.*

3.4.2 Distribution

3.4.2.3 Dermal Exposure

COMMENT: Page 95: line 9: Although absorption of exposure through the skin is apparently less that orally, but I did not see where the document addressed that relative amount of 2,4-D that might be available to be absorbed through this tissues. My sense is that under most human exposure condition, dermal contact is more common. Perhaps this this be addressed if my assumption is correct. Even if I am wrong, others may have my incorrect view.

RESPONSE: *A sentence was added indicating that since dermal absorption occurs, the distribution of 2,4-D into tissues is probably similar to that observed in oral animal studies.*

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

COMMENT: Page 97, line 19: Maybe should note that there are elimination studies in humans, they just are not restricted to inhalation.

RESPONSE: *Text was added indicating that 2,4-D has been measured in the urine of workers exposed to 2,4-D by a combination of routes, including inhalation.*

3.5.1 Pharmacokinetic Mechanisms

Metabolism

COMMENT: Page 108, line 32: Are some bacteria able perform some metabolism? Are these bacteria that reside in humans? If so, is that a possible route for occurrence of metabolites?

RESPONSE: *Studies of metabolism of 2,4-D suggest that 2,4-D is not broken down, which would rule out a role for bacteria. It is excreted mostly unchanged and a small portion conjugated. There is no information regarding breakdown products being identified in the urine. There is, however, environmental biodegradation (Chapter 6).*

3.5.2 Mechanisms of Toxicity

COMMENT: Page 111, line 26: Given behavioral alterations in animals, the findings from the Agricultural Health Study that depression was slightly increased among applicators exposed to 2,4-D and slightly decreased among spouses (neither statistically significant) may be of interest.

Beseler C, Stallones L, Hoppin JA, Alavanja MCR, Blair A, Keefe T, Kamel F. Depression and pesticide exposures in female spouses of licensed pesticide applicators in the Agricultural Health Study. J Occup Environ Med 2006; 48:1005-1013.

Beard JD, Hoppin JA, Richards M, Alavanja MCR, Blair A, Sandler DP, Kamel F. Pesticide exposure and self-reported depression among wives in the Agricultural Health Study. Environ Res 2013; 126:31-42.

RESPONSE: *Information from both studies was added to the profile.*

3.7 CHILDREN'S SUSCEPTIBILITY

COMMENT: Page 116, line 14: Do the differences in handling and clearance of 2,4-D among dogs, rodents, and humans provide any useful information regarding concern for exposures among children, or other susceptible groups?

RESPONSE: *Yes, and this is mentioned in Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE.*

COMMENT: Page 116, line 17: Is "conclusive" the only level of evidence that can be considered, or should something like the IARC scale of possible, probable, and sufficient be useful to convey concern to the public? In this case does "not conclusive" here mean there is no concern?

RESPONSE: *"Conclusive" was changed with "convincing."*

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

COMMENT: Page 118, line 1: Although this may occur for various compounds, is this possible with 2,4-D? Since it is largely un-metabolized, it would seem that no other chemical would affect any biologic measurement of exposure. Maybe this should be mentioned here.

RESPONSE: *The comment refers to standardized text in Section 3.8, BIOMARKERS OF EXPOSURE AND EFFECT. This text appears in all profiles; it is not specific for 2,4-D.*

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

COMMENT: Page 119, line 4: Should probably quantify "recent."

RESPONSE: *Within days was added.*

COMMENT: Page 119, line 25: A phrase like this is needed because, at least, application exposure often only occurs for a few hours out of the day and the range can be quite large.

RESPONSE: *No response is necessary.*

COMMENT: Page 120, line 10: Might want to include information from a study with 2,4-D measurements among applicators in Iowa and NC (Thomas K et al. J Expo Sci Environ Epidemiol 2010; 20(2):119-134, although they did not develop a pharmacokinetic model.

RESPONSE: *Thomas et al. (2010) was included in the profile.*

COMMENT: Page 120, line 11: I am not sure you can make the statement that exposure for "farm family" members is due to direct exposure. Some direct exposure may occur, but for many spouses and most children it is more likely to be due to indirect exposure (contamination of surfaces, drift from application areas, in house hold dust) than direct exposure.

RESPONSE: *The Reviewer's statement about direct exposure was added to the text.*

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

COMMENT: Page 121, line 27: Need to be clear here. Were there no studies on this point, or did all of the studies show no susceptibility?

RESPONSE: *Text was clarified; no studies on this point were identified.*

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

COMMENT: Page 124, line 24: Some of the general population can be exposed from 2,4-D in house dust. This is more likely in areas where the chemical is used agriculturally, but 2,4-D has been found in homes elsewhere also.

RESPONSE: *House dust was added to the text.*

COMMENT: Page 124, line 27: I am not sure this statement is correct. The absorption rate may be higher through the GI than other routes, but whether this results in "more" exposure through the GI partially depends upon the concentration of chemical available to the different locations. This probably differs considerably by who is being exposure, i.e., manufacturers, applicators, or the general population.

RESPONSE: *The comment refers to the sentence: "There is no evidence suggesting that the toxicity of 2,4-D is route-specific, but considerably more 2,4-D is absorbed through the gastrointestinal tract than through the skin." To avoid confusion, the second part of the sentence was deleted.*

COMMENT: Page 124, line 34: House dust?

RESPONSE: *House dust was added.*

Figure 3-5. Existing Information on Health Effects of 2,4-D

COMMENT: Page 125: This chart might be a little deceptive. Many epidemiologic studies do not fit into one of the route of exposure categories because they probably have exposure from all routes.

RESPONSE: *ATSDR agrees with the Reviewer, but this is standard presentation in profiles.*

3.12.2 Identification of Data Needs

Chronic-Duration Exposure and Cancer.

COMMENT: Page 127, line 26: If "conclusive" is the only standard you can apply to human studies this comment is okay, but if a suspicion that is not conclusive is possible. You need to be clear what the stance is here.

I note that below the "conclusive" standard is not applied to animal or mechanistic studies. Associations that are "possible" are discussed in that light.

RESPONSE: *The word conclusive was changed with convincing.*

COMMENT: Page 127, line 29: The comment refers to the following sentence: "As is not uncommon with epidemiological studies, limitations encountered in these studies include unreliable exposure assessment and simultaneous exposures to other chemicals." If simultaneous exposure to other factors is not allowed for epidemiology studies to be useful, then clearly none would meet this standard. Multiple exposures is the human condition. Given the long history of "useful" epidemiologic studies, I think the simultaneous exposure standard is detrimental to assembling useful information.

RESPONSE: *Simultaneous exposure makes it more difficult to make statements regarding individual chemicals as opposed to classes of chemicals (i.e., herbicides, organophosphorus pesticides, organochlorine pesticides, etc.).*

COMMENT: Page 127, line 29: The comment refers to the following sentence: "As is not uncommon with epidemiological studies, limitations encountered in many of these studies include unreliable exposure assessment and simultaneous exposures to other chemicals." This statement indicates that "many" epidemiologic studies suffer from these two limitations. This implies that not all do. Can they be used to draw a conclusion other than "not conclusive." Or do you actually mean all studies have these limitations?

RESPONSE: *The word many was deleted. As stated above, the word conclusive was replaced with the word convincing.*

COMMENT: Page 127, line 30: I do not think the assumption that manufacturing workers are only exposed to the active ingredient. In order to make this recommendation this assertion must be documented with monitoring or other information on lack of exposures in the manufacturing situation.

RESPONSE: *The text was reworded as follows: "It seems prudent, however, to continue to monitor populations exposed to 2,4-D, such as pesticide applicators and manufacturers."*

Genotoxicity

COMMENT: Page 128, line 9: Also cite: Hou L, Andreotti G, BaccarelliAA, Savage S,Hoppin JA, Sandler DP, Barker J, Zhu AA, Hoxha M, Dioni L, Zhang X, Koutros S, Beane Freeman LE, Alavanja MC. Lifetime pesticide use and telomere shortening among male pesticide applicators in the Agricultural Health Study. Environ Health Perspect 22013; 121:919-924.

RESPONSE: *Both studies were added.*

COMMENT: Page 128, line 18: There are a lot of studies listed. I think you need to be clear about which show positive links and which do not, or at least provide some indication of how "mixed" these results are.

RESPONSE: *The main purpose of this section is to indicate whether or not studies are available, not to repeat the results of individual studies. The words "mixed results" were changed with "both positive and negative results" results, further explanation seems unnecessary.*

COMMENT: Page 128, line 21: I do not understand this conclusion. Above both positive and negative studies are listing and these provide "mixed" results, yet the conclusion is no additional information would be useful.

RESPONSE: *Please see response to comment below.*

COMMENT: Page 128, line 22: I do not understand this conclusion. Above both positive and negative studies are listing and these provide "mixed" results, yet the conclusion is no additional information would be useful. However, since there clearly are no, or very few, humans with exposure only to 2,4-D, I guess this final sentence is okay, if the only studies of value are those of individuals that have only one exposure. Another approach would be to recognize that all humans have multiple exposures (tobacco, alcohol, air pollution, multiple occupational exposures, household chemicals, etc.) that are not experimentally controlled and that it would be important to know the effects of 2,4-D in this mix. It is possible to design studies to deal with possible confounding.

RESPONSE: *The last sentence in the paragraph stating that additional studies are unnecessary was changed with the Reviewer's suggestion that efforts to design studies to deal with possible confounding should be encouraged.*

Reproductive Toxicity

COMMENT: Page 128, line 25: Again I wonder if it would be helpful to have more categories of evidence than 1) conclusive and 2) not conclusive?

RESPONSE: *Conclusive was changed to convincing.*

COMMENT: Page 128, line 31: This summation seems a little odd. There is some evidence of reproductive toxicity but it is not conclusive. Yet the summary says additional studies are not needed.

RESPONSE: *The text was revised to indicate that additional studies in animals do not seem necessary. Only one animal reported reproductive effects. Joshi et al (2012) reported alterations in sperm parameters in rats dose wit 50 mg 2,4-D/kg by gavage (non-relevant mode of exposure for humans exposed to 2,4-D) for 30 days.*

Immunotoxicity

COMMENT: Page 129; line 23: There are epidemiologic findings linking 2,4-D with various asthmatic conditions. See comments above.

RESPONSE: *The study regarding asthma (Hoppin et al. 2008) was added to the respiratory section and the one regarding rheumatoid arthritis (De Roos et al. 2005) was added to the immune section.*

Epidemiological and Human Dosimetry Studies.

COMMENT: Page 130; line 22: It is not clear what is the intent of this statement. Does it mean that significant associations in human studies can never imply causality? If not never, then when? Perhaps there should not be a blanket statement that seems to cover all human studies.

RESPONSE: *This is the response to the same comment provided earlier by the Reviewer: The statement about a significant association not necessarily implying causality was felt necessary for those who mistakenly assume that a statistically significantly increased risk means that exposure to the chemical caused the adverse health outcome. It does, however, indicate that exposure to the chemical plays a role in the health outcome, and as the number of studies showing statistically significant associations increase, so does the biological plausibility or the weight of evidence. This was added to the document.*

COMMENT: Page 130; line 24: I think a better statement would be to conduct studies in areas where exposures to 2,4-D and other chemicals in the workplace can be adequately characterized and not imply that exposures to chemicals other than 2,4-D are largely absent in the manufacturing industry.

RESPONSE: *The statement suggested by the Reviewer was added to the text.*

Biomarkers of Exposure and Effect.

COMMENT: Exposure; Page 130, line 30: It is not clear what is recommended here. If you have urinary measures, why would you want to estimate exposure? Does exposure mean dose to specific tissues? If so, that would be valuable, but it does not make sense to go backward from a biologic measure of the chemical to a estimate of external exposure.

RESPONSE: *As indicated in Section 3.8.1, 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 such a reference concentration (RfC) or reference dose (RfD).*

COMMENT: Effect; Page 131, line 2: Having a 2,4-D specific effect would be valuable, but if this is not possible, would studies to more fully understand the relationship between exposure, measured blood or urine levels, and biologic effects be of value? Availability of biologic effects for other hazardous chemicals have proven useful even when the effect is not restricted to a specific chemical (as most effects are not).

RESPONSE: *The text in the section is in accordance with ATSDR's guidelines. The type of studies that the Reviewer suggests certainly would provide valuable information. However, the purpose of this section is to state whether or not a there is a unique health effect or group of effects (i.e., a syndrome) that can be attributed to exposure to 2,4-D. The answer is no.*

Comparative Toxicokinetics.

COMMENT: Page 131, line 30: Would additional information on OAT1 activity by age, sex, health and other conditions be of value to help characterize acceptable exposures for susceptible populations?

RESPONSE: *Text provided in the comment by the Reviewer was added to the profile: "Studies of OAT1 activity by age, sex, health, and other conditions would be of value to help characterize acceptable exposures for susceptible populations."*

3.12.3 Some Ongoing Studies

COMMENT: Page 133: I am a little worried that this list is too incomplete. I think there are pesticide studies in Canada, France, Norway, New Zealand that might provide information on 2,4-D and there are probably others. It is okay if you do not make it sound comprehensive.

RESPONSE: *ATSDR is limited to searching for ongoing research studies in the RePorter database, which lists federally funded research. However, if the reviewer can provide ATSDR with specific data regarding ongoing research on 2,4-D, it could be included in this section.*

COMMENT: Page 133, line 13: Machael Alavanja has retired.

RESPONSE: *The text was revised as suggested by the Reviewer.*

5.3 USE

COMMENT: Page 148, line 5: Should there be a comment about the expected increase in use of 2,4-D because of newly developed genetic modified crops that can tolerate it?

RESPONSE: *A statement addressing the potential increase in use due to the development of genetically modified crops was added to the profile.*

6.2.2 Water

COMMENT: Page 154, line 10: I did not find the reference, but I think there are studies of measurements of 2,4-D in surface water from studies in Iowa.

RESPONSE: *Surface water monitoring data for Iowa were located in a 2014 document prepared by the Iowa Department of Natural Resources Water Quality Bureau. It's unclear if this is the study mentioned by the reviewer. However, the document is a draft version, which cannot be cited. When the finalized document is published, ATSDR can add these data to Section 6.4.2. Section 6.2.2 discusses releases to water, not necessarily monitoring studies. Section 6.4.2 discusses monitoring data in surface water, groundwater, and drinking water.*

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

COMMENT: Page 167, line 16: I think you should provide the monitoring information and cite the studies directly, rather than a review of them. Then make your determination of what is the exposure level of the general population

RESPONSE: *ATSDR has changed the reference to CDC (2015), which shows the National Health and Nutrition Examination Survey (NHANES) levels in urine samples for a representative sample of the general U.S. population.*

COMMENT: Page 176, line 11: Should describe urinary measurements for IA and NC farmers also.

> Thomas K, Dosemeci M, Hoppin JA, Sheldon L, Croghan C, Gordon S, Jones M, Reynolds S, Raymer J, Akland G, Lynch C, Knott C, Sandler DP, Blair A, Alavanja MCR. Urinary biomarker, dermal and air measurement results for 2,4-D and chlorpyrifos farm applicators in the Agricultural Health Study. J Exposure Sci Environ Epid 2010; 20(2):119-134.

RESPONSE: *ATSDR has added data from the requested study.*

COMMENT: Page 177, line 5: Study in IA with multiple measurements found 2,4-D in the urine occurred in the winter indicating exposure occurred in times when application was not occurring. The findings for atrazine, another herbicide, did not show this pattern.

> Bakke B et al. Exposure to atrazine and selected non-persistent pesticides among corn farmers during a growing season. J Exp Sci Environ Epidemiol 2009; 19(6):544-554.

RESPONSE: *ATSDR has added data from the requested study.*

6.6 EXPOSURES OF CHILDREN

COMMENT: Page 179, line 11: Analysis of 2,4-D levels in hour dust provides information about possible exposure.

> Colt JS et al. Comparison of pesticide levels in carpet dust and self reported pest treatment practices in four US sites. Highest levels were in IA. Colt JS et al. Comparison of pesticide levels in carpet dust and self-reported pesticide treatment practices in four US sites. J Expl Anal Environ Epidemiol 2004; 14:74-83.

RESPONSE: *ATSDR has added data from the requested study.*

MINIMAL RISK LEVEL (MRL) WORKSHEET

COMMENT: Page 238: Is this a standard procedure. Just seems odd to drop the two highest categories.

RESPONSE: *Dropping the highest dose to improve the fit in benchmark modelling is an acceptable procedure since the main interest is in the region of inflection of the curve that identifies the benchmark dose (BMD) and lower confidence limit on the BMD (BMDL). However, if the data set consist only of a few dose levels, then dropping high doses would not be appropriate.*

APPENDIX B. USER'S GUIDE

COMMENT: Page 243: At several places in the document it is noted that results in dogs were excluded because dogs were more sensitive than humans and rodents. How does that square with this statement?

RESPONSE: *The comment refers to the following statement that appears in Appendix B, USER'S GUIDE: "If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels."*

Results in dogs were not excluded from the profile, but were not considered for MRL derivation because of dog's unusually long half-life elimination of 2,4-D from plasma (about 10-fold that of humans), as explained in the response to an earlier comment. This results in substantially higher body burdens, and thus, increased susceptibility to 2,4-D. The statement in Appendix B means (and perhaps should be clarified) that, all other things equal between species, the most sensitive will be selected.

Comments provided by Reviewer #2:

Overall quality and comprehensiveness of the draft document

COMMENT: The overall quality, comprehensiveness of the document is very good. Available data through early 2015 is included and summarized well, although briefly. I am aware of recent unpublished data on very young children's exposures to 2,4-D that are not included, but since these data are not yet peer-reviewed, they are not appropriate for inclusion in the current profile.

RESPONSE: *No response is necessary.*

COMMENT: General issues regarding children's health relevant to 2,4-D toxicology are adequately discussed in the document.

RESPONSE: *No response is necessary.*

Chapter 1: Public Health Statement

COMMENT: The statement overall is properly aimed at the intended audience, namely the lay public. It is written in an appropriate, relatively informal style, and presents relevant information in a non-technical way.

RESPONSE: *No response is necessary.*

COMMENT: The answers to the questions appear to address the concerns of the audience adequately. The summary statements are, by and large, consistent and supportable.

RESPONSE: *No response is necessary.*

COMMENT: Scientific terms that may be difficult for the intended audience to understand are noted in the manuscript. See the attached summary of page and line-by-line suggestions and the associated highlighted lines.

RESPONSE: *Responses for the line-by-line suggestions are provided below.*

Chapter 2: Relevance to Public Health

COMMENT: The stated health effects known to occur in humans as results of exposures to high levels of 2,4-D are adequately addressed. An area in which more information would be desirable, but not currently accessible in the published, peer-reviewed literature, is the area of potential adverse health effects, including developmental effects, that may be of concern in young children, especially those of preschool ages, including infants and children of ages below five years. A few studies have been done that include measurements of 2,4-D in human breast milk, but this information has not shown up yet in the published scientific literature.

RESPONSE: *Comprehensive literature searches are conducted before the post-public version of the profile is developed. It is possible that those searches will identify some of the reports that the Submitter refers to. However, if the Submitter becomes aware of the publication of studies on this issue, ATSDR would appreciate the Submitter notifying the Agency.*

COMMENT: The document does a good job pointing out data needs overall, for example, neurobehavioral effects from low chronic doses. There is not adequate human data for chronic oral exposure of the mother that may lead to expression of 2,4-D in human breast milk, although the animal studies suggest that this transmission occurs.

RESPONSE: *No response is necessary.*

COMMENT: Most jargon is avoided, which helps to make this document more accessible to the average, although scientifically informed, reader. However, acronyms throughout need to be defined in the text; sometimes the first time they occur, and sometimes later in the text when a large amount of other text has intervened. This would help the reader.

RESPONSE: *The Reviewer's suggestion was followed by defining acronyms the first time they appear in a major section.*

COMMENT: The adverse health effects observed in laboratory animals, including mice, rats, dogs and other species, have generally been at extremely high doses, usually acute durations. Although these effects provide needed information on the mechanisms of toxicity and the affected organs and behaviors, they are unlikely to occur in humans, except in the case of accidental or intentional poisoning.

RESPONSE: *No response is necessary.*

COMMENT: The exposure conditions for the animal experiments are mostly well described. For a few experiments, more information should be included on the duration of the experiments. This is highlighted in the document.

RESPONSE: *The following response was provided for the line-by-line comment below: According to ATSDR's Guidance for Preparation of Toxicological Profiles, excessive study details should be avoided in Chapter 3 in favor of bottom-line statements, if possible. However, if, for example, two intermediate-duration studies report seemingly inconsistent results in the same animal species and at the same dose level, the exposure-duration would be explicitly indicated because it could explain the different results.*

Chapter 3: Health Effects

<u>Toxicity—Human Studies</u>

COMMENT: Adequately designed human studies are identified in the text, and both their strengths and limitations are discussed. The authors' conclusions are addressed and presented with appropriate assessments of the strengths and weaknesses.

RESPONSE: *No response is necessary.*

COMMENT: NOAELs and LOAELs are included if available.

RESPONSE: *No response is necessary.*

COMMENT: Appropriateness of the statistical tests used in the various studies cannot be evaluated for most of the studies listed, with the information given in the document. The number of human studies at the low levels of exposure expected in most environments is relatively small.

RESPONSE: *No response is necessary.*

COMMENT: I am not aware of additional studies that are important and should be included in this profile.

RESPONSE: *No response is necessary.*

Toxicity—Animal Studies

COMMENT: Adequately designed animal studies are identified in the text, and both their strengths and limitations are discussed.

RESPONSE: *No response is necessary.*

COMMENT: Given that the animal studies described pinpointed the toxicity mechanisms in a large number of species, including a more sensitive species (dog), with similar results in target organ identification and mode of action, but with toxic levels differing in the species, it seems that experimentation with other animal species should not be a priority. There appears to be sufficient animal information to lead scientists to an approximate good understanding of 2,4-D toxicity in humans.

RESPONSE: *No response is necessary.*

COMMENT: From the information presented in the text, the conclusions drawn by study authors seem to be accurately presented.

RESPONSE: *No response is necessary.*

COMMENT: Not all studies determined NOAELs or LOAELs. Of those that did, the information presented is adequate.

RESPONSE: *No response is necessary.*

Levels of Significance Tables and Figures

COMMENT: Comments are indicated in the accompanying document, Specific comments by page and line number, and are highlighted in the draft profile.

RESPONSE: *Responses to the line-by-line comments are provided below.*

COMMENT: The categorization of Less Serious and Serious adverse effects in the LSE tables is ok.

RESPONSE: *No response is necessary.*

Evaluation of Text

COMMENT: See specific comments by page number and line.

RESPONSE: *Responses to the line-by-line comments are provided below.*

Toxicokinetics, Mechanisms of Action, and Neuroendocrine Aspects of Toxicity

COMMENT: These sections are is organized appropriately into human and animal information. Well-presented discussions of the processes, mechanisms, and target organs are included. Human-animal differences are discussed here, as well as in preceding sections. The possible neuroendocrine effects are discussed carefully.

RESPONSE: *Responses to the line-by-line comments are provided below.*

Children's Susceptibility and Other Susceptible Populations

COMMENT: The important differences between adults and children in terms of physical, behavioral, and developmental characteristics that may make children more susceptible to adverse health effects from toxics exposures are well summarized.

RESPONSE: *No response is necessary.*

COMMENT: The document addresses briefly other populations that may be highly susceptible to toxic exposures: elderly, immunocompromised, and those with other morbidities.

RESPONSE: *No response is necessary.*

Biomarkers of Exposure and Effect

COMMENT: Since 2,4-D is not metabolized, but is rather excreted unchanged primarily in urine and perhaps other body fluids, an additional biomarker of exposure is not needed. Biomarkers of effect consist of those measureable compounds in blood, saliva, exhaled air, sweat, urine, and feces that indicate adverse effects on target organs, such as (and most prominently) effects on kidney function, such as

creatinine or albumin. However, these chemicals do not specifically represent damage from 2,4-D exposure, but instead can arise from several other toxic chemicals to which an individual may be exposed, or indeed from degenerative processes in the human body that occur with age and disease. This topic is adequately addressed in the document.

RESPONSE: *No response is necessary.*

Treatments for Toxic Effects

COMMENT: Section not reviewed in depth. It seems adequate for the purposes of the profile.

RESPONSE: *No response is necessary.*

Adequacy of the Database

COMMENT: This section discusses several areas in which there is a large amount of data available in the open, published literature. Most areas of possible exposure/effects do not appear to need additional research to provide data on 2,4-D, and the conclusions are appropriate. However, some animal studies, mostly in rats, have indicated changes in maternal behavior after birth of offspring, and have also indicated cross-placental transfer of 2,4-D, and low concentrations of 2,4-D in animal breast milk. Human studies directed at concentrations of 2,4-D in human neonates and in breast milk are warranted, especially because infants may be extremely susceptible to toxic insults, as discussed earlier.

RESPONSE: *Text was added to the Developmental Effects section in the Data Needs Section stating that data on levels of 2,4-D in breast milk and in neonates born to women exposed to 2,4-D through farming activities would be valuable.*

Chapter 4: Chemical and Physical Information

COMMENT: See page and line-specific comments, marked in document.

RESPONSE: *ATSDR has addressed comments from this section including converting density from English to metric units.*

Chapter 5: Production, Import/Export, Use and Disposal

COMMENT: This information is adequately presented.

RESPONSE: *No response is necessary.*

Chapter 6: Potential for Human Exposure

COMMENT: By and large, this chapter traces 2,4-D from point of release to the environment, its path through the environment, and suggests the route of human exposure (primarily dermal or inhalation routes) in acute exposures. Because residues deposited on surfaces can be transferred into the home

environment through track-in, on clothing, pets, etc. the potential exists for chronic exposure in the home or work environment.

RESPONSE: *ATSDR has added data regarding track-in potential from homeowners, children, and pets to the indoor home.*

COMMENT: The text adequately covers relevant information on transport, partitioning, transformation, and degradation of 2,4-D. Background levels in environments not associated with hazardous waste sites and pesticide handling and manufacture are also adequately covered. Units of measurement are not always consistent and are sometimes missing. Spots in the text where this is particularly egregious are highlighted in the draft.

RESPONSE: *ATSDR has provided converted units in parentheses where the Reviewer has highlighted them in the text.*

COMMENT: An additional route of exposure in humans is through ingestion of foodstuff that contains relatively small concentrations of 2,4-D, which could possibly lead to chronic exposures of concern. Ingestion of intentional large toxic doses in adults does not seem to be of major concern, based on few reported cases of death or high morbidity from ingestion of pesticides containing high percentages of 2,4-D. Because most levels in foods are very low, relative to measured toxic doses, ingestion by adults does not appear to be of great concern. However, exposures of young children through the dermal route and subsequent ingestion, or ingestion in neonates and in infants through diet and breast milk, are of concern. Especial concern is warranted for young children in the families of workers who handle pesticides, or who work at or near Superfund sites. This concern is addressed in the document, particularly for farm families and for families of pesticide and waste site workers. Additional research on exposures of neonates and young children is needed and justifiable.

RESPONSE: *ATSDR has added this suggestion as a data need to Section 6.8.1.*

Chapter 7: Analytical Methods

COMMENT: Available analytical methods for determination of 2,4-D in environmental media and in foods and beverages including drinking water, as well as in urine are available and summarized. These methods are mostly sufficient for current use and do not require additional methods development. To examine further possible exposures of human neonates and infants to 2,4-D and its relatives, precise and accurate methods for determination of these compounds at low levels in breast milk are needed. The challenges in developing such methods are both collection of sufficient quantities of milk, and overcoming the high fat content and difficulties in handling of the milk during analysis.

RESPONSE: *ATSDR has added this as a potential data needs to Section 7.3.1.*

Chapter 8: Regulations and Advisories

COMMENT: The summaries in this chapter seem complete. It would be useful and informative here and in the preceding chapters to give some explanation as to why EPA and other US government agencies have determined that 2,4-D is not carcinogenic to humans, whereas IARC has categorized it as a possible

human carcinogen (2B)—in the same category as the known carcinogenic polycyclic aromatic hydrocarbons, such as benzo[a]pyrene. This explanation however brief, is especially needed.

RESPONSE: *As indicated in previous responses, IARC recently published Monograph 113 online. This is now mentioned throughout the profile. It is not ATSDR's practice to mention carcinogenicity classifications in the text of Chapter 8. However, Table 8-1 was revised to indicate that EPA classified 2,4-D in Group D and that IARC (2016) placed 2,4-D in Group 2B. EPA has not determined that 2,4-D is not carcinogenic; the Agency determined that there are no adequate data either to support or refute human carcinogenicity.*

Chapter 9: References

COMMENT: The reference list appears complete through mid-2015. A link to one additional reference that could be included is provided in the list of specific comments by page and line number.

RESPONSE: *The reference in question is Morgan et al. (2015), which now appears in Section 6.5, General Population and Occupational Exposure.*

Unpublished Studies

COMMENT: I am not aware of any unpublished studies that are available for detailed comment at this time.

RESPONSE: *No response is necessary.*

Comments provided by Reviewer #2 (Continued):

*The page numbers given here are the numbers of the pages in the entire document, starting with the title page as Page 1. The numbered lines of concern in the text are highlighted.

1. PUBLIC HEALTH STATEMENT FOR 2,4-D

COMMENT: Page 25 (of 250) line 8-9: Although 2,4-D is not highly absorbed through the skin, amounts remaining on the skin after contact can easily be transferred to the mouth, other body parts, and other surfaces. This could result in "second-hand" exposures which may be especially important for children.

RESPONSE: *Text suggested by the Reviewer was added to the section.*

COMMENT: Page 26, line 25-34: It might be useful for the general public to have these recommended limits expressed in English as well as metric units, or at least in some easily understood concentrations. This deserves a bit of explanation, because it seems inconsistent with the vast majority of references previously cited.

RESPONSE: *The Reviewer probably means lines 15-27, which state guidelines and regulations for 2,4-D in environmental media and workplace air developed by various government agencies. The units for 2,4-D in water or air are in metric units, which is how the agencies list them in the published documents. It is unclear what the Reviewer means by this being inconsistent with the vast majority of references previously cited.*

2.2 SUMMARY OF HEALTH EFFECTS

COMMENT: Page 33, line 26-27: This deserves a bit of explanation, because it seems inconsistent with the vast majority of references previously mentioned and cited in the document.

RESPONSE: *The comment refers to IARC's recent classification of 2,4-D as "possibly carcinogenic to humans." The text was revised to indicate that IARC recently published the full assessment online. The revised text also states the basis for IARC decision to place 2,4-D in Group 2B.*

2.3 MINIMAL RISK LEVELS (MRLs)

Oral MRLs

COMMENT: Page 36, line 10: Gestation Days. Need to define acronym first time used in text.

RESPONSE: *The acronym was defined.*

COMMENT: Page 36, line 25: Need to define first time used in text.

RESPONSE: *The acronym was defined.*

3.2.2.2 Systemic Effects

Respiratory Effects

COMMENT: Page 50, line 7-8: Although intermediate duration is defined earlier in this document, it would be informative to the reader in these cases to give the durations of exposure for each of these intermediate-duration studies.

RESPONSE: *According to ATSDR's guidance, excessive study details should be avoided in Chapter 3 in favor of bottom-line statements, if possible. However, if, for example, two intermediate-duration studies report seemingly inconsistent results in the same animal species and at the same dose level, the exposure-duration would be explicitly indicated because it could explain the different results. No changes were made.*

Hepatic Effects

COMMENT: Page 54, line 30: Changed sentence, which was not clear as written.

RESPONSE: *The sentence was revised as follows: "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)."*

Endocrine Effects

COMMENT: Page 56, line 29: Units?

RESPONSE: *Units were added (mg/kg/day).*

3.2.2.7 Cancer

COMMENT: Page 67, line 17: Classified based on what?

RESPONSE: *Based on inadequate evidence in humans and limited evidence in experimental animals, this was added to the text.*

3.3 GENOTOXICITY

In vivo Exposure Studies

COMMENT: Page 85, line 2: Full citation now available?

RESPONSE: *The text was revised by replacing Andreotti et al. (2014) with full papers by Hou et al. (2013) and Andreotti et al. (2015). Corresponding changes were made to Table 3-4.*

3.4 TOXICOKINETICS

COMMENT: Page 87, lines 10 and 30: Reference?

RESPONSE: *Line 10 is in the brief overview at the beginning of the toxicokinetics section. Traditionally, no references are included in this section. Line 30 is in Section 3.4.1.2, Oral Exposure and states the following: "Results from studies in volunteers have shown that oral absorption of 2,4-D in humans is rapid and virtually complete." The specific references and results of the studies that support the introductory sentence are immediately below.*

3.4.2.4 Other Routes of Exposure

COMMENT: Page 93, line 9: Define PND first time in text.

RESPONSE: *The suggestion was followed.*

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

COMMENT: Page 100, line 1: Define HQ first time in text.

RESPONSE: *The definition of Hazard Quotient was put in parenthesis.*

Human and Rat (Durkin et al. 2004)

COMMENT: Page 102, line 5: Cite reference for equation.

RESPONSE: *The Henderson-Hasselbach equation is a common equation described in any general chemistry textbook, a specific reference is not needed.*

COMMENT: Page 102, line 29: Is this an appropriate listing of an unpublished industrial report?

RESPONSE: *The comment refers to a citation that appears as follows in the PBPK section: (Smith et al. 1980, unpublished). It is appropriate to cite unpublished industrial reports when the original report could not be obtained. The word "unpublished" was deleted. The reference in Chapter 9 indicates that it was cited in Durkin et al. (2004).*

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

COMMENT: Page 115, line 33: Is NHANES specifically defined earlier. If not, define acronym here.

RESPONSE: *The acronym was defined earlier in Section 3.2.3.2.*

3.11 METHODS FOR REDUCING TOXIC EFFECTS

COMMENT: Page 118, line 32: Be careful to spell out numbers below 10 when not used with specific units. Applies throughout the text.

RESPONSE: *The word "one" was changed by number 1. Numbering throughout the profile follows ATSDR's guidance.*

3.11.2 Reducing Body Burden

COMMENT: Page 119, line 11: Clarify. Volume of urine/min increased with increased pH, but did the concentration of 2, 4-D in the urine remain the same?

RESPONSE: *No information was provided in the original study cited by Roberts (Prescott et al. 1979, Brit. J. Clin. Pharmacol. 7:111-116).*

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

COMMENT: Page 136, Table 4-3: The units are inconsistent in this table, e.g., for density, as they are in following Table 4-4. Provide metric, not just English, units.

RESPONSE: *ATSDR has also provided the density of 2,4-D sodium in metric units.*

Figure 6-1. Frequency of NPL Sites with 2,4-D Contamination

COMMENT: Page 146, Figure 6-1: What is meant by "frequency" in this chart? This is not at all clear. Does it mean there are, for example, two contaminated sites in each of the states shaded green, and one contaminated site in each of the states shaded red? Needs clarification for the reader.

RESPONSE: *No change was made based upon this comment. Frequency of occurrence is the correct phrasing.*

6.2 RELEASES TO THE ENVIRONMENT

6.2.2 Water

COMMENT: Page 148, line 32: concentration?

RESPONSE: *No change was made based upon this comment. The sentence in question represents an application rate not a measured concentration. Water concentrations post-application are discussed in Section 6.4.2.*

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

COMMENT: Page 151, line 6: Correct, but confusing, since 2,4-D can be transported to water both directly and indirectly.

RESPONSE: *ATSDR has reworded the sentence to read: 2,4-D is released to water both from direct application for weed control, and through unintentional processes such as spray drift and runoff.*

6.3.2.2 Water

COMMENT: Page 154, line 25: Define EHE first time used.

RESPONSE: *No change was made based on this comment. EHE was defined in Section 6.1.*

COMMENT: Page 155, line 11: Define DCP first time used.

RESPONSE: *No change was made based upon this comment. DCP was defined on the previous page.*

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

6.4.1 Air

COMMENT: Page 158, line 13: Range of concentrations? Mean/median?

RESPONSE: *No change to the profile was made based upon this comment. Only 1 out of 880 samples contained 2,4-D. Thus range, mean, median etc., are not particularly relevant.*

COMMENT: Page 159, line 3: Deposition could be better assessed for comparison with other data if put in ug/m^2-day.

RESPONSE: *No change to the profile was made based upon this comment. These were the units reported in the citation and are consistent with the way that concentrations are reported in the paragraph.*

6.4.2 Water

COMMENT: Page 160, line 11: Inconsistent units. Put amount applied in English units (lb/acre) to agree with area treated (in acres).

RESPONSE: *ATSDR has added the appropriate conversion for English units.*

6.4.3 Sediment and Soil

COMMENT: Page 162, line 4: Clarify!

RESPONSE: *ATSDR has changed the sentence to reflect that Vertisol is a soil order: In soil samples collected from one uncultivated and one cultivated California Vertisol soil, 2,4-D concentrations ranged from 8 to 143 ppb at the uncultivated site and was not detected at the cultivated site (Graham et al. 1992).*

6.4.4 Other Environmental Media

COMMENT: Page 162, lines 21-25: Give these concentrations in metric units also, e.g. 0.00169 ppm (0.00169 ug/g) for comparison purposes. Are the three decimal places quoted for some of these numbers really significant, based on the detection limits of the analytical procedures used?

RESPONSE: *ATSDR has also given the units ppm as (ug/g) and reported the numbers as in the citation.*

COMMENT: Page 163, line 16: Show metric units.

RESPONSE: *ATSDR has also supplied the metric units.*

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

COMMENT: Page 163, line 32: Additional analysis of the exposures of 121 adults in the CTEPP study is reported in Morgan, MK, Int J Hyg Environ Health, 2015: 479-88. doi 10:1016/j.ijheh.2015.03.015

RESPONSE: *ATSDR has added data from this study.*

COMMENT: Page 171, lines16-17: Screwy units! Concentrations/L of urine? Total weight excreted in urine over the collection period per person? Weight excreted per kg body weight?

RESPONSE: *No change to the profile was made based upon this comment. It is the amount of 2,4-D in all the urine of each subject excreted over a 4-day period.*

COMMENT: Page 171, lines 24-25: In this study? Shown how?

RESPONSE: *ATSDR has replaced "significant" with important to avoid confusion with statistically significant.*

6.8.2 Ongoing Studies

COMMENT: Page 178, line 16: Include years for NHANES IV.

RESPONSE: *NHANES is an ongoing study; therefore, no change was made to the profile based upon this comment.*

Comments provided by Reviewer #3

COMMENT: This document summarizes available information on the characteristics, exposure, and health effects of 2,4-dichlorophenoxyacetic acid (2,4-D). It is prepared in the standard format for the ATSDR Toxicological Profiles, and discusses health effects in relationship to route of exposure (inhalation, oral, dermal).

RESPONSE: *No response is necessary.*

COMMENT: Overall, the document is well written and well documented. Tables and Figures are good comprehensive compilations of information, and are useful for comparison of data and values. At difference with similar documents, this one is not overly repetitious, though at times the requested format leads to some redundancy. A number of issues may need additional corrections and/or clarifications, as suggested in the specific comments below. Furthermore, some sections would benefit of an overall conclusion statement.

RESPONSE: *No response is necessary.*

COMMENT: Specific comments are listed below; they are divided by chapters, with indications of the page number and line.

RESPONSE: *No response is necessary.*

CHAPTER 1. PUBLIC HEALTH STATEMENT

COMMENT: The intended audience for this chapter is the lay public, and this chapter is written in a simple and direct style, perhaps even too simple in some instances. All major information on 2,4-D is summarized in a simple and clear manner.

RESPONSE: *No response is necessary.*

COMMENT: Page 1, lines 30-34: It is indicated that the t1/2 of 2,4-D in air is 19 h. The next sentence indicates that 2,4-D breaks down in soil very quickly with a t1/2 of 6 days. Perhaps this paragraph can be slightly rephrased.

RESPONSE: *ATSDR has changed the wording to indicate that 2,4-D is not persistent in soil.*

COMMENT: Page 2, line 9: Many herbicidal products (instead of many herbicides) contain 2,4-D.

RESPONSE: *ATSDR has made the suggested change.*

COMMENT: Page 3, lines 3-7: Is it necessary to have these in bullet points?

RESPONSE: *ATSDR feels that presenting this information in bullets makes it easier for the reader to detect, as opposed to being presented within the text of the paragraph.*

COMMENT: Page 3, lines 32-33: The discrepancy between the EPA evaluation and the recent IARC evaluation is evident, and may lead to questions and concerns. Yet neither here nor in the whole document is this issue satisfactorily discussed. If the classification has only been announced and the full document has not been released yet, this should be stated.

RESPONSE: *A full report of IARC's rationale for its recent classification of 2,4-D recently became available, and this was added to the text, along with the basis for the classification (Section 2.2). ATSDR only presents the classifications of the agencies and the agencies' basis for doing so, but does not discusses why there may be discrepancies.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

COMMENT: Page 8, line 1: It would be useful to insert the structure of 2,4-D here or to refer to Fig. 4-1 on p. 111.

RESPONSE: *ATSDR has referred to Table 4-1, which has the chemical structure of 2,4-D.*

COMMENT: Page 9, section 2.2: Should there be an initial statement about acute toxicity (LD_{50})?

RESPONSE: *Information regarding effects due to acute high exposure in humans are mentioned starting on line 23. Of course, there are no LD_{50} data in humans. The estimated lethal amounts (converted to doses assuming a 70 kg body weight) mentioned on page 9, lines 27-34, are in the range of the animal LD_{50}values, if humans had ingested pure 2,4-D, which was not the case. LD_{50} data in animals were included on page 11, lines 12-14.*

COMMENT: Page 9, line 29: How reliable is this estimate by Nielsen (1965)? How does it compare with animal studies?

RESPONSE: *Please see previous response above.*

COMMENT: Page 10, third paragraph: An additional study that could be mentioned is Woods JS et al. J Natl Cancer Inst. 1987; 75: 899-910.

RESPONSE: *Woods et al. (1987) was added to the profile. However, the study does not provide specific information for 2,4-D. From Woods et al. (1987) page 902: "Further evaluation of the risk of NHL observed among forestry herbicide sprayers with respect to specific chemicals used and duration of exposures indicated that all forestry sprayers reported the combined use of 2,4-D and 2,4,5-T as well as various commercial herbicide preparations containing these and other chemicals."*

COMMENT: Page 13, lines 24-27: The discrepancy between EPA and IARC evaluations, which needs to be addressed.

RESPONSE: *Please see response to comment on page 3, lines 32-33, above.*

COMMENT: Page 15, from line 23 on: It should be made clear that 2,4-D is given to the mother and that pups are presumably exposed only through the milk. Thus, the measured effect is in the pups (decreased body weight) but the dose is given to the dam. Using this type of data set for extrapolation of MRL is not common, to my knowledge, and may want to be better explained.

RESPONSE: *It is generally understood that when gestational exposure or lactational exposure of offspring is mentioned it means that the mothers were treated. This type of data set (generally developmental studies) has been commonly used for MRL derivation by ATSDR and for RfD and RfC derivation by EPA. In fact, inspection of the Integrated Risk Information System (IRIS) database shows that EPA has derived 37 RfDs/RfCs based on developmental endpoints; ATSDR has derived 29 MRLs based on developmental end points.*

COMMENT: Page 15, line 5, 6: The fact that a 100-fold UF was applied may be indicated here.

RESPONSE: *Normally, the uncertainty factors are mentioned after the point of departure has been identified. In this case, page 20, lines 19-20.*

COMMENT: Page 21, last paragraph: The study chosen for deriving the intermediate MRL leads to this apparent paradox of an intermediate MRL which is 22-fold lower than the chronic MRL. Perhaps this needs to better discussed.

RESPONSE: *It is not an uncommon occurrence that a developmental end point is more sensitive than a systemic end point (i.e., liver, kidney, cardiovascular effect). Because developmental studies are of acute or intermediate durations, MRLs based on developmental end points are likely to be lower than MRLs based on a chronic study for the same chemical and, as a result, a chronic-duration MRL is not derived. However, the intermediate-duration MRL is protective of chronic exposures.*

CHAPTER 3. HEALTH EFFECTS

Section 3.1 INTRODUCTION

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

COMMENT: In this, as in the following sections, I will comment only when I disagree with some statements or have suggestions to change text etc. In the absence of any specific comments, I agree with the writing, the choices and the conclusions.

RESPONSE: *No response is necessary.*

COMMENT: Page 25, line 9, 10: Could it be stated here that the overall NOAEL is 100 mg/m^3? Will Table 3-1 and Figure 3-1 be inserted here? If not it should be said that they are at the end of the document.

RESPONSE: *LSE Tables 3-1, 3-2, and 3-3 and Figures 3-1 and 3-2 are inserted in the corresponding sections (inhalation, oral, or dermal) in the final document. It is correct that the overall NOAEL for the study was 100 mg/m^3, but it is not appropriate to indicate this in the standardized text on lines 9-10. It happens that for 2,4-D there was only one inhalation study available so the standardized text had to be modified accordingly.*

COMMENT: Page 27: Some repetitions, related to the presence of NOAEL values for different end-points in Table 3-1 and Figure 3-1, may be corrected.

RESPONSE: *It is not clear what the Reviewer means by correcting NOAELs in Table 3-1 and Figure 3-1. The table and figure were developed in accordance with ATSDR's guidance.*

COMMENT: Page 28, line 10: Define "organic" (e.g., other organic compounds).

RESPONSE: *It was meant to be "organic solvents" such as benzene, toluene, bromopropane, etc. The word solvent was added.*

COMMENT: Page 29: An interesting case report on human 2, 4-D poisoning may be added (Berwick P., JAMA 1970; 214: 1114-1117).

RESPONSE: *Information from Berwick (1979) was added to the profile.*

COMMENT: Page 30: It would be useful here and in the following pages to clearly separate acute from intermediate, and chronic studies. In particular the range for intermediate studies is very large (15–364 days), and encompasses traditional sub-acute and sub-chronic studies. At times a one-year study is also considered chronic. I would suggest specifying the duration of the study when reporting the effects.

RESPONSE: *Acute, intermediate, and chronic studies are normally discussed in separate paragraphs when there are several studies for each duration. According to ATSDR's guidance, excessive study details should be avoided in Chapter 3 in favor of bottom-line statements, if possible. However, if, for example, two intermediate-duration studies report seemingly inconsistent results in the same animal species and at the same dose level, the exposure-duration would be explicitly indicated because it could explain the different results. Studies' durations are indicated in the LSE Tables.*

COMMENT: Page 42: A study with oral administration of 2,4-D may be worth mentioning and commenting on (Squibb RE et al. Neurobehav Toxicol Teratol. 1983; 5: 331-335).

RESPONSE: *Information from Squibb et al. (1983) was added to the profile.*

COMMENT: Page 47, lines 15-19: Need to discuss the IARC conclusions.

RESPONSE: *As mentioned above, a full report of IARC's rationale for its recent classification of 2,4-D recently became available, and this was added to the text along with the basis for the classification (Section 2.2).*

COMMENT: Page 53, line 11: A study by Mattsson JL et al. (Neurobehav Toxicol Teratol 1986; 8: 255-263) found no evidence of neurotoxicity of 2,4-D given dermally to rats for three weeks. There is also a series of studies by Schulze GE et al., all from 1988, dealing with the neurotoxicity of 2,4-D-n-butylester, though the route of administration was subcutaneous.

RESPONSE: *Mattson et al. (1986) tested a 12% solution of the dimethylamine salt. However, as stated in Section 3.1, INTRODUCTION, this profile discusses only 2,4-D and simple salts (e.g., sodium, potassium, ammonium).*

COMMENT: Page 55: This section seems to be the one in which to discuss the EPA classification of 2, 4-D and the recent classification by IARC as a possible human carcinogen (2b).

RESPONSE: *This section summarizes cancer data in humans. The EPA and IARC classifications were added at the end of the section.*

COMMENT: Page 55, lines 18-20: This sentence (In final…) needs to be rewritten.

RESPONSE: *The comment refers to a summary of results in a study by McDuffie et al. (2001). The text was revised for clarity.*

Section 3.3 GENOTOXICITY

COMMENT: This section presents all available in vivo and in vitro data on genotoxicity, and as often is the case, results from genotoxicity studies provide contrasting results, with both positive and negative findings. The overall conclusion related to the potential genotoxicity of a compound must rely on a weight-of-evidence approach, which, as indicated in the document, is quite difficult in this case, even applying the usual considerations (e.g., *in vivo* vs. *in vitro*, prokaryotic systems vs. mammalian cells, etc.). The two Tables are useful.

RESPONSE: *No response is necessary.*

Section 3.4 TOXICOKINETICS

COMMENT: This section is very comprehensive as it discusses a large number of studies on absorption, metabolism, distribution, and excretion after exposures of different species through different routes.

RESPONSE: *No response is necessary.*

COMMENT: Page 68, line 12: This section belongs to elimination rather than absorption, and indeed is repeated later on p. 78.

RESPONSE: *The comment refers to the following sentence: "However, the area under the curve (AUC), elimination half-life, maximal concentration, and time of maximal concentration were significantly greater in females than in males (Griffin et al. 1997a)." This sentence appears in the section on absorption by the oral route. The sentence was deleted in this section.*

COMMENT: Page 75, Fig. 3-3: It should be made clear that the metabolites shown derive from conjugation reactions which favor elimination, and that there is no evidence that metabolism, which is minimal, contributes to toxicity.

RESPONSE: *A sentence was added at the end of section 3.3, Metabolism, stating that conjugation, although minimal, favors elimination in the urine. That metabolism does not contribute to toxicity is mentioned in Section 3.5.1, Pharmacokinetic Mechanisms, under Metabolism.*

COMMENT: Page 77, line 10: A summary paragraph could be added here before the PBPK section.

RESPONSE: *A brief summary is presented in the overview at the beginning of Section 3.4, TOXICOKINETICS. An additional summary seems unnecessary.*

Section 3.5 MECHANISMS OF ACTION

COMMENT: Page 84: The initial section on pharmacokinetic mechanisms is interesting as it introduces some aspects that where not covered in the previous section devoted to this topic.

RESPONSE: *No response is necessary.*

COMMENT: Page 85, line 31: The section on mechanism of toxicity focusses exclusively on biochemical changes measured in brain. Yet the central nervous system does not appear to be a primary target for 2, 4-D. Is there any information on biochemical/molecular effects of 2, 4-D in other organs, such as liver of kidney? For example, is there enzyme induction? Is this relevant?

RESPONSE: *Little is known about the mechanism(s) of action of 2,4-D. The Reviewer is correct in that the central nervous system does not appear to be a primary target in humans or adult animals. However, it should be kept in mind that treatment of rat dams with 2.5 mg 2,4-D/kg/day during lactation resulted in in significantly reduced body weight in the offspring during the first weeks of life (Stürtz et al. 2010). This low LOAEL was used to derive an MRL for 2,4-D. The maternal 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, so a summary of the studies by Evangelista de Duffard and coworkers is relevant.*

The only information on enzyme induction is presented in Section 3.9, which states that 2,4-D was found to increase the expression of some CYP1 cytochromes in rat liver, kidney, and mammary gland and of some microsomal enzymes in the liver of mice and rats, and decrease some phase II enzymes in rat liver. This, however, suggests that the toxicity of chemicals that are metabolized by the affected enzymes may increase or decrease by co-exposure to 2,4-D depending on whether metabolism produces a reactive intermediate or a detoxification product, but this does not tell much about the mechanism of toxicity of 2,4-D.

Possible general mechanisms of action for 2,4-D and related chemicals that have been explored mostly in studies in vitro include effects associated with the plasma membrane, interference with cellular metabolic pathways involving acetylcoenzyme A, and uncoupling of oxidative phosphorylation (Bradberry et al. 2000).

COMMENT: Page 88, line 4: This paragraph and the whole section do not seem necessary as the concept has been discussed earlier.

RESPONSE: *The comment refers to Section 3.5.3, Animal-to Human Extrapolations. This section is part of all Toxicological Profiles, so even though some of this information has been mentioned earlier in the document, it also needs to be presented here.*

Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

COMMENT: Page 90, line 9: The indicated EPA studies appear to be very relevant and should be discussed in more detail.

RESPONSE: *The text was revised to include more details of the EPA studies cited.*

Section 3.7 CHILDREN'S SUSCEPTIBILITY

COMMENT: Page 91: This introductory section on the blood brain barrier is very interesting and informative, though not much relevant for 2,4-D.

RESPONSE: *The comment refers to standardized text that appears in all profiles.*

COMMENT: Page 93, line 14: There is no real conclusion on whether children are or are not expected to have special susceptibility. Yet, as indicated the MRL is derived for a developmental study, and the young appear to have a diminished elimination rate for 2, 4-D which may enhance toxicity. Perhaps this needs to be better discussed.

RESPONSE: *This is mentioned in Section 3.10, Populations that are Unusually Susceptible.*

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

COMMENT: Page 95, line 27: Although discussed later (p. 99), it could be added here that the pH of urine leads to significant changes in 2,4-D urinary levels.

RESPONSE: *A sentence was added stating that urinary pH is an important determinant of urinary levels of 2,4-D.*

COMMENT: Page 96, line 31: Is this paragraph needed? Its significance is unclear.

RESPONSE: *The comment refers to Section 3.8.2, Biomarkers Used to Characterized Effects Caused by 2,4-D. Yes, the section is needed. The point being made is that the effects induced by exposure to 2,4-D are not unique to 2,4-D, so they do not necessarily indicate exposure to 2,4-D.*

Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

COMMENT: Page 97, line 8: This information on enzyme induction could be moved to the section on mechanisms of toxicity.

RESPONSE: *As stated above, 2,4-D altering the activity of phase I or phase II metabolic enzymes suggests that the toxicity of chemicals that are metabolized by the affected enzymes may increase or decrease depending on whether metabolism produces a reactive intermediate or a detoxification product, but it does not tell much about the mechanism of toxicity of 2,4-D.*

COMMENT: Page 97, line 13: These studies are not very informative. However, it may be stated that given that 2,4-D exposure could coexist with exposure to other pesticides, more information on potential interactions would be useful.

RESPONSE: *The statement suggested by the Reviewer was added to the text.*

Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

COMMENT: Page 97, line 33: It could be mentioned here that dogs, which have a lower clearance, experience higher susceptibility to 2, 4-D toxicity, as evidenced by the lower NOAEL values (Table 3-2).

RESPONSE: *Text was added to specify that higher susceptibility has been shown in dogs.*

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

COMMENT: Page 98, line 17: It is unclear why reference is made here to generic textbooks of clinical toxicology. Could they eventually be listed in the references?

RESPONSE: *This is part of the format of the profiles. The textbooks are mentioned because they provide specific information on 2,4-D. The authors of specific chapters that provide information regarding 2,4-D are listed in the reference section.*

Section 3.12 ADEQUACY OF THE DATABASE

COMMENT: This section contains a further summary of all aspects discussed in previous sections, and emphasizes those aspects which would benefit of further data. I agree with most considerations. Neurotoxicity and developmental neurotoxicity studies may be carried out with 2, 4-D, utilizing EPA guidelines.

RESPONSE: *Studies by Squibb et al. (1983), Marty et al. (2013), and Mattson et al. (1997) provide adequate information on neurotoxicity and developmental neurotoxicity. However, it would be reassuring if the findings of Stürtz et al. (2010) of developmental effects in rat offspring from dams*

treated with low doses of 2,4-D during lactation could be replicated by other groups of investigators This was added to the subsection on Developmental Effects in section 3.12.

COMMENT: Page 109: It would seem that none of the ongoing studies addresses any of the identified data needs.

RESPONSE: *The identification of ongoing studies is limited to the RePORTER database.*

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

COMMENT: Acceptable as is.

RESPONSE: *No response is necessary.*

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

COMMENT: Acceptable as is.

RESPONSE: *No response is necessary.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

COMMENT: The title is a bit misleading because the first half or more of this section is devoted to a discussion of environmental fate of 2,4-D. This initial part is well done and comprehensive. The second part focusses on human exposure and body burden.

RESPONSE: *ATSDR will consider changes to chapter titles in future profiles.*

COMMENT: Page 143, line 19: The data presented here and in Tables 6-2 and 6-3 are very interesting and useful, but there is only limited and somewhat confusing discussion of their significance. Are there significant differences related to age? Are there any temporal trends? How do the urinary levels reported in the NHANES study compare to those of workers involved in the production or use of 2,4-D and to those of their families? Some discussion of these issues would be very useful, to put all data in perspective.

RESPONSE: *ATSDR has added a sentence indicating that urinary levels of 2,4-D have generally remained static over the temporal period shown. ATSDR has added another study that discusses the relationship of urinary 2,4-D levels and population age and sociodemographic factors.*

COMMENT: Page 144, line 2: The sentence continues on p. 151.

RESPONSE: *No changes were made to the profile since it is unclear what this comment means.*

COMMENT: Page 155, line 13: Again, here is an opportunity to discuss whether children may have a higher body burden.

RESPONSE: *ATSDR has added language to indicate that slightly greater levels are observed in children versus adults.*

COMMENT: Page 156, line 4: This provides the opportunity to better discuss body burden of workers vs. the general population.

RESPONSE: *ATSDR has added language comparing NHANES data to urinary 2,4-D levels of applicators.*

COMMENT: Page 157, line 29: Typo, "general".

RESPONSE: *ATSDR corrected the typo.*

CHAPTER 7. ANALYTICAL METHODS

COMMENT: This chapter contains a comprehensive description of methods; I have no further comments.

RESPONSE: *No response is necessary.*

CHAPTER 8. REGULATIONS AND ADVISORIES

COMMENT: It is interesting and reassuring that the intermediate-duration oral MRL derived from the Stürtz et al. (2010) study, is almost identical to the RfD derived by the EPA from an unpublished sub-chronic study in rats.

RESPONSE: *No response is necessary.*

CHAPTER 9. REFERENCES

COMMENT: There is a very comprehensive list of references. A few additional ones have been suggested in previous sections.

RESPONSE: *Pertinent suggested references were added to the profile.*

CHAPTER 10. GLOSSARY

COMMENT: This chapter is useful and comprehensive.

RESPONSE: *No response is necessary.*

APPENDIX A. ATSDR MINIMAL RISK LEVEL AND WORKSHEETS

COMMENT: This section is very well presented and very useful, as it clearly describes the study used to derive the MRL, showing the most relevant end-point, and explaining the considerations and the process utilized to arrive at the MRL value.

RESPONSE: *No response is necessary.*

APPENDIX B. USER's GUIDE

COMMENT: This is very useful to understand and use the large Tables (e.g. 3-2)

RESPONSE: *No response is necessary.*

APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

COMMENT: Always very useful

RESPONSE: *No response is necessary.*

UNPUBLISHED STUDIES (IF APPLICABLE TO REVIEW)

COMMENT: None known.

RESPONSE: *No response is necessary.*

ADDITIONAL COMMENTS

COMMENT: 2, 4-D was a component of Agent Orange together with 2,4,5-T. The latter was found to be contaminated with TCDD, while 2, 4-D is not. Any need to mention this somewhere in the document, given its "historical" value

RESPONSE: *The profile was revised to include this information in Section 5.3. It was noted that 2,4-D is sometimes confused with 2,4,5-T, which at one point was contaminated with TCDD. However, mentioning in the profile that 2,4-D was a component of Agent Orange would not be appropriate. Since it was 2,4,5-T that was contaminated with TCDD, which was the chemical responsible for the health effects, mentioning that 2,4-D was in Agent Orange may give the reader the impression that it may have been responsible for the adverse effects.*