DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR NITROPHENOLS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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Agency for Toxic Substances and Disease Registry

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Peer reviewers for the third pre-public draft of the Toxicological Profile for Nitrophenols were:

Rémi Béranger, PhD
Epidemiologist, midwife
University hospital of Rennes (Brittany, France)
Research Institute for Environmental and Occupational Health, Inserm (France)

Kurunthachalam Kannan, PhD
Department of Pediatrics
550 First Avenue, MSB6-698
New York University School of Medicine
New York, NY 10016, USA

Satoshi Inomata, ScD
National Institute for Environmental Studies
16-2 Onogawa, Tsukuba, Ibaraki 305-8506, JAPAN

NOTE: Peer reviewer comments are written next to "COMMENTS:" in unformatted text. Any italicized text following the comment is added for clarification purposes. Any page and line numbers that were added by the Reviewers have been kept, but often will not align with the appropriate text.

Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses

Additional general comments provided by Reviewer #1 outside of charge questions:

COMMENT: I appreciate the opportunity to review an excellent report. This is a very thorough review of environmental occurrence, fate, toxicity, human exposure of 2-,3-, and 4-nitrophenols. The presentation is very clear and interpretation of data are critical and reasonable. Despite the fact that these compounds continue to be produced and used, there are limited studies on human exposure and toxicity. I have provided some minor comments to enhance the significance and clarity of the presentations for each of the chapters below. Some of the overall comments are listed below.

- It is worth pointing out that there are only 13 reliable toxicological studies that have described effects of nitrophenols and all except Tang et al. 2016 and Li et al. 2016/2017, are >20 years old. This point needs to be mentioned in chapter 1.
- Tables and figures were presented well and captures all the data concisely.
- There is no study describing early life stage exposures and effects (children); multigenerational rodent studies are limited – transplacental transfer, accumulation in tissues and organs following exposure to these chemicals are not understood.
- Chapters 4 and 5 can be moved to chapters 2 and 3.
- Relative potencies of three nitrophenols can be assessed through in vivo and in vitro studies for critical toxicological end points. For example, one would question if all 3 nitrophenols are equally toxic or they differ in their potency.

RESPONSE: *Although we have not added the specific number of toxicological studies into Chapter 1, there is an existing sentence in Chapter 1 that reads as follows: "The existing experimental animal database is limited regarding the health effects of nitrophenols, because for many endpoints there are only a small number of well-conducted studies." This sentence addresses the point that there are very few reliable toxicological studies that have described effects of nitrophenols. Additionally, in response to this comment, we have also updated the sentence to address the age of the literature. The updated sentence reads as follows: "The existing experimental animal database is limited regarding the health effects of nitrophenols, because for many endpoints there are only a small number of well-conducted studies, and the majority of the literature is over 20 years old."*

Regarding the organization of Chapters 4 and 5 being moved to Chapters 2 and 3, the organization of the profile is a standard format that exists for all newly updated ATSDR toxicological profiles. Thus, these suggested changes are unable to be made.

A sentence was added to Section 6.2 (Identification of Data Needs) regarding a need for more research on the relative potencies of the three nitrophenols: "Additional research on the relative potencies of 2-, 3-, and 4-nitrophenol would also add to the health effects literature."

Reviewer #1 did not provide point by point answers to the charge questions, but instead provided one overall comment for each set of questions on a chapter. ATSDR responded to these overarching comments where appropriate.

Chapter 1. Relevance to Public Health

COMMENT: The chapter is clear and covers major references related to nitrophenols toxicity. Major toxicological endpoints of nitrophenols have been reviewed critically. The number of reliable studies are limited and those studies including exposure conditions have been adequately described. Exposure doses are high and no sub-chronic and low level exposure studies are available. Based on the limited data, MRLs have not been derived for any of the toxicological endpoints and it is justified.

RESPONSE: *No revisions were suggested.*

COMMENT: When the animal studies are mentioned, specifying doses (at least a range) would enhance the value. Also, as a conclusion/summary (P6), it is important to state that these doses are several orders of magnitude higher than the predicted doses of exposure in human populations. It is also important to indicate that many chronic toxic end points (e.g., endocrine effects – changes in hormones) or early life stage effects (i.e., vulnerable populations) were not examined and this data gap/knowledge gap should be identified. I agree with the conclusion that it is premature to derive the MRL value with little toxicological data available and majority of the studies are high dose acute exposures. The chapter can include some additional reasons why the MRL could not be derived.

RESPONSE: *In general, when studies are mentioned in the text, the key dose information is mentioned in tandem, particularly the NOAEL and LOAEL (if applicable), as well as the study duration, and animal species, and any observed health effects (particularly when studies are mentioned in the text in Chapter 2). However, the study information presented in Chapter 1 is meant to assist in providing a high-level summary of the health effects observed in the body of literature. More specific information about every dose administered in each study is contained within the LSE tables, which can be found in Table 2-1, Table 2-2, and Table 2-3 for inhalation, oral, and dermal studies, respectively.*

A sentence was added to Chapter 1 explaining that doses administered in animal studies are expected to be higher than doses experienced by humans. This additional sentence reads: "The sensitive endpoints observed in animal studies are at dose levels that are relatively high compared to those that may be experienced by human populations."

Additionally, a sentence was added to Chapter 1 that points out the inadequate characterization of many chronic toxic endpoints and early life stage health effects in the current literature. This added sentence reads as follows: "Additionally, many chronic toxic endpoints as well as early life stage health effects have not been adequately characterized in the currently available literature."

In general, the purpose of Chapter 1 with respect to the introduction of the MRLs is to state whether any MRLs have been derived, and if so, what they are specifically. More details about the MRL derivation process and reasons for why they have or have not been derived are generally reserved for the larger descriptions of the MRL derivations in Appendix A. The following sentence found in Chapter 1 explains that more information about why no MRLs have been derived can be found in Appendix A: "The rationale for not deriving each MRL is discussed in greater detail in Appendix A." As such, no further changes were made to the profile based on this comment.

COMMENT: P1, Lines 13-14: "They also have low vapor pressures, which could allow global dispersion via precipitation (Harrison et al. 2005)". This statement can confuse readers as low vapor

pressures depict low volatility and therefore less amenable for atmospheric transport and therefore global dispersion is not expected. I would rephrase the statement as "They also have low vapor pressures, and therefore potential for long range atmospheric transport is low"

RESPONSE: *This comment refers to the following sentence in section 1.1 (Overview and U.S. Exposures): "They also have low vapor pressures, which could allow global dispersion via precipitation (Harrison et al. 2005)." The sentence has been rephrased as requested, and now reads: "They also have low vapor pressures, and therefore potential for long range atmospheric transport is low."*

COMMENT: P2, Lines 23-25: I would rephrase the sentence to increase clarity: "However, respiratory effects were not consistently observed, as both in an intermediate-duration (>14 –364 days) inhalation study (Hazleton 1983) and an intermediate-duration oral study (Koizumi et al. 2001), no significant effects were reported." Can be rephrased as "However, no significant respiratory effects were observed, both in an intermediate-duration (>14 –364 days) inhalation study (Hazleton 1983) and an intermediate-duration oral study (Koizumi et al. 2001)."

RESPONSE: *This comment refers to the following sentence in section 1.2 (Summary of Health Effects – Respiratory Effects): "However, respiratory effects were not consistently observed, as both in an intermediate-duration (>14 –364 days) inhalation study (Hazleton 1983) and an intermediate-duration oral study (Koizumi et al. 2001), no significant effects were reported." The sentence has been rephrased as requested, and now reads: "However, no significant respiratory effects were observed, both in an intermediate-duration (>14 –364 days) inhalation study (Hazleton 1983) and an intermediate-duration oral study (Koizumi et al. 2001)."*

COMMENT: P3, Lines 1, 4, 6: "intermediate inhalation study" and "intermediate oral studies" can be confusing. It should be "intermediate duration inhalation study". If you like to avoid extra wording 'duration' throughout at least this need to be mentioned as 'intermediate study' means 'intermediate duration'. This info is available on P10 (chapter 2), which is after this page and readers who just read chapter 1 may not know what intermediate inhalation or intermediate oral refers to.

RESPONSE: *This comment refers to the use of the following phrases throughout the profile: "intermediate inhalation study," "intermediate dermal study," and "intermediate oral study." Changes were made as requested throughout the profile to "intermediate-duration inhalation/oral/dermal study."*

COMMENT: P4 and P5, Figures 1-1 and 1-2: These figures do not really depict any respiratory or hematological effect, expect in 1-1, where methemoglobin is shown at middle dose. It is important to list respiratory and additional hematological effects on these two figures

RESPONSE: *This comment refers to Figures 1-1 and 1-2 in section 1.2 (Summary of Health Effects). Figure 1-1 and Figure 1-2 depict all respiratory and hematological effects observed in the available literature. Although there are only minimal endpoints listed in these figures, they represent the extent of all respiratory/hematological endpoints observed, and are shown corresponding to the lowest dose/concentration where the described health effects have been observed. Thus, no additional changes to Figure 1-1 or Figure 1-2 have been made based on this comment.*

COMMENT: P6: On this page, I would also mention that the exposure doses studied in animal studies are several orders of magnitude higher than typical human exposure doses. Because this chapter is on public health relevance, a sentence or two on this regard is needed. This will also justify why a MRL at this stage could not be derived.

RESPONSE: *This comment refers to section 1.3 (Minimal Risk Levels (MRLS)). In response to this comment, the following sentence was added: "The sensitive endpoints observed in animal studies are at dose levels that are relatively high compared to those that may be experienced by human populations."*

COMMENT: P7, P8; Figures 1-3 and 1-4: the title indicates "Targets". It is more accurate to state that these are end points. Instead of "Targets", it is preferable to replace that with "End points". This change is also needed in the text. Targets are normally used for 'organs', not health end points. These two are different.

RESPONSE: *This comment refers to Figures 1-3 and 1-4 in section 1.3 (Minimal Risk Levels (MRLS)). The titles of these figures are specified by the ATSDR profile development guidance document[1]. As such, we are unable to change the titles to use the term "End Points" instead of "Targets". Thus, no changes were made to the profile based on this comment.*

Chapter 2. Health Effects

COMMENT: This chapter has described human and animal toxicology studies in detail. Among the 13 relevant/reliable animal toxicology studies, majority of them have used very high doses of exposure. Very few of them have used appropriate dose response relationships. Majority of the studies were conducted >20 years ago. NOAEL and LOAELs have been identified for those studies that have reliable exposure and outcome measures and used rat models for toxicity testing. I suggest that the critical NOAEL and LOAEL values from important studies be highlighted in the report. Alternatively, a short summary at the end of the chapter would add value. After reading the document, it would help the readers to know what would be the most critical value (lowest dose that resulted in an effect), at least a ballpark estimate. The categorization of LOAELs as 'serious' and 'less serious' has been defined well and therefore it is appropriate that this categorization has been made. Little discussion is made in terms of mechanisms of toxic effects and that is probably due to the fact that original investigations were not focused on the mechanistic link.

RESPONSE: *Critical NOAEL and LOAEL values from important studies are generally highlighted where relevant. All subsections of Chapter 2 begin with a short summary of the evidence, followed by specific information from each study containing relevant information on the particular health endpoint of interest. Additionally, all NOAEL and LOAEL information from each well-conducted study for each health endpoint of interest is presented in Table 2-1, Table 2-2, and Table 2-3, as well as graphically in Figure 2-2 and Figure 2-3. These tables and figures give a comprehensive overview of all health endpoints with notable effects, the doses at which these effects occurred, and also key information about the study design. Figure 1-1 and Figure 1-2 present a graphical representation of the lowest doses that result in an effect, as well as the effects associated with those particular doses and the duration associated with each critical effect. As all of the requested information is currently contained within the profile in places generally reserved for summarizing this type of information, no changes were made to the profile based on this comment.*

COMMENT: P59, Line 11- P60, line 16: Reproductive effects listed under 'other' category: Several studies, Li et al. 2006, Li et al. 2009 and Zhang et al., 2013, 2015, 2016, 2017 and Mi et al., 2013 present a very important finding of reproductive effects at low doses of exposure and appears to be a reliable study, and the effects observed are much more significant than observed 'ocular' effects. It is prudent to

[1] https://www.atsdr.cdc.gov/toxprofiles/guidance/profile_development_guidance.pdf

describe these studies in detail and derive a NOAEL/LOAEL values for reproductive effects of 4-nitrophenol. Although the report identifies lack of corroboration from oral, inhalation and dermal exposure studies, these studies are important and cannot be ignored.

RESPONSE: *The aforementioned studies listed in the Reproductive effects section have been described in detail in the text. However, it is ATSDR policy to focus on studies with routes of exposure that are relevant to ways in which humans could potentially be exposed to the chemical; intraperitoneal injection of nitrophenols is not a typical exposure scenario for humans. Given the importance of the findings in these studies, they have been included in the text of the profile, however, they do not fit the criteria used to include studies in the LSE tables or figures since they do not use a relevant route of exposure. Thus, the studies are only described in the text, and a note of caution has been included since the animals were exposed through a route that is not typical of human exposures. No changes were made to the profile based on this comment.*

COMMENT: The oral gavage study of Koizumi et al. 2001 (table 2-2, #12) reports 10/12 M and 10/12 F rats died at a dose of 1000 mg/kg/day. Despite this NOAEL values for several other end points were reported as 1000 mg/kg/day. This appears to be odd; if 83% of rats died at 1000 mg/kg/day, there is no explanation for how NOAEL for other end points can be that value. The other study (##11 on the table 2-2) also reported significant mortality at 230-320 mg/kg/day dose.

RESPONSE: *These NOAELs at 1,000 mg/kg/day for other health end points indicate that rats did not exhibit significant changes in these health effects at that level of exposure. Although the majority of rats died at 1,000 mg/kg/day, these rats did not exhibit significant bodyweight loss prior to death, as an example. No changes were made to the profile based on this comment.*

COMMENT: For some of the studies, it is important to describe experimental conditions. For instance, inhalation exposure – how was this done. Did they use inhalation chamber? Did they measure air concentrations? How did they produce nitrophenol vapors for inhalation exposure? Some of these experimental conditions may have some deficiencies that need to be identified.

RESPONSE: *There are three main inhalation studies cited in the profile, Smith et al. (1988), Hazleton (1983), and Hazleton (1984). For each of these three studies, experimental conditions such as how they produced nitrophenol vapors, how they measured air concentrations, and if the study used inhalation chambers were added to the profile in the first mention of these studies in Section 2.2 (Death) in response to this comment. For Smith et al. (1988), the following language was added to the profile: "In both of these studies conducted by Smith et al. (1988), 4-nitrophenol dust was generated using a 3-stage glass dust generator containing 4-nitrophenol sodium salt (composed of 75% 4-nitrophenol, sodium salt, and 25% water), with atmospheric concentrations taken at 30 or 60 minute intervals using three sampling ports at nose-level in the inhalation chamber." For Hazleton (1983), the following language was added to the profile: "This study generated 4-nitrophenol dust using Wright dust-feed mechanisms that fed dust into exposure chambers using a turret that mixed the dust with air. Nominal chamber concentrations were measured each time new test material was measured, and three gravimetric samples were taken from each chamber each day." For Hazleton (1984), the following language was added to the profile: "Exposures were conducted in glass and steel inhalation chambers, with chamber concentrations of 2-nitrophenol vapors measured at least twice per day. 2-nitrophenol vapors were produced in the study by sweeping the headspace of a glass generation flask containing melted 2-nitrophenol into the inhalation chambers."*

COMMENT: P36, lines 11-18: Koizumi study has a better design with a real dose-response at least to determine a meaningful LD50 value for 4-nitrophenol and this can be highlighted a bit more in details on

this page. On line 20, it would benefit the readers to make some statement with regard to an LD50 value. It appears that a dose above 400 mg/kg/day results in higher deaths in laboratory rodents.

RESPONSE: *In general, it is ATSDR practice not to infer an LD50 from a study unless an LD50 is specifically calculated and presented in the study results. Although the Koizumi et al. (2001) study did produce death at higher levels of oral exposure to 4-nitrophenol, the study authors did not calculate an LD50 from their results. Thus, we are unable to provide an LD50 from this study. No changes were made to the profile based on this comment.*

COMMENT: P39, line 21: The statement "The 5 mg/m3 dose is considered the NOAEL for respiratory effects in this study" This is a critical information, not presented in Figure 2.2 but presented in Table 2.1. I think this is a key information that may be presented in overall summary or chapter 1. I would suggest that lowest values found to have an effect on various toxicological end points may need to be presented somewhere in summary.

RESPONSE: *The P39 Line 21 specifies that the 5 mg/m^3 concentration that is considered the NOAEL for respiratory effects from Hazleton (1984) does not show up in Figure 2-2. This was an accidental omission from this figure. The 5 mg/m^3 NOAEL is now present in the updated version of Figure 2-2. With respect to the presentation of the lowest values found to have an effect on various toxicological endpoints, this information is presented graphically in Figure 2-2 and Figure 2-3, as well as in Figure 1-1 and Figure 1-2 in Chapter 1. Thus, no changes were made to the profile based on this portion of the comment.*

COMMENT: P11, Line 24: "targets" is used again, but "end point" is more appropriate. If there is a reason for using "target" instead of "end point" a justifiable explanation is needed.

RESPONSE: *This comment refers to the following sentence in section 2.1 (Introduction): "Research on the health effects of nitrophenols suggest several sensitive targets of toxicity." The word "targets" has been changed to "endpoints," and the sentence now reads: "Research on the health effects of nitrophenols suggest several sensitive endpoints of toxicity."*

COMMENT: Figure 2-2: Without reading Table 2-1, it is not clear what the number in front of "R" refers to? E.g., 1R, 2R, 3R and 4R (they related to each study presented in Table 2-1, but an explanation in the figure 2-2 legend will improve clarity.

RESPONSE: *With respect to the meaning of the number in front of "R" in Figure 2-2, explanations exist both in the corresponding Table 2-1, as well as in the Appendix D User's Guide. Additionally, the information contained within the legends for the LSE figures is standardized as defined by ATSDR. As such, no changes were made to the profile based on this comment.*

COMMENT: Table 2-2: "FI" is not found in the table legend.

RESPONSE: *"FI – food intake" was added to the table legend.*

COMMENT: Table 2-3: "DX and RX" are not found in table legend.

RESPONSE: "*DX – developmental toxicity" and RX – reproductive toxicity" were added to the table legend.*

COMMENT: Page 35, Line 15: "in Smith et al. " should be changed to "(Smith et al. 1988);"

RESPONSE: *This comment refers to the following sentence in section 2.2 (Death – Inhalation): "Acute inhalation exposure to 4-nitrophenol in rats at levels up to 4,059 mg/m3 for 4 hours produced no*

mortality in Smith et al. (1988).” The reference format has been changed: “Acute inhalation exposure to 4-nitrophenol in rats at levels up to 4,059 mg/m3 for 4 hours produced no mortality (Smith et al. 1988).”

COMMENT: Page 36, Line 7 : Reference formatting issue that needs to be corrected “(Branch 1983)” should be (Branch, 1983).

RESPONSE: *This comment refers to a reference formatting issue in section 2.2 (Death – Oral): “Death was observed in 4 of 5 male Sprague-Dawley rats after a single gavage dose of 268 mg/kg, and in 3 of 5 Sprague-Dawley female rats after a single gavage dose of 171 mg/kg ((Branch & Stout, 1983)).” The reference format has been fixed: “Death was observed in 4 of 5 male Sprague-Dawley rats after a single gavage dose of 268 mg/kg, and in 3 of 5 Sprague-Dawley female rats after a single gavage dose of 171 mg/kg (Branch and Stout 1983b).”*

COMMENT: P 41, Line 15: “significance of these findings are unclear” ‘are’ should be “is”

RESPONSE: *This comment refers to the following sentence in section 2.4 (Respiratory – Other): “The toxicological significance of these findings are unclear.” The word “are” has been changed to “is,” and the sentence now reads: “The toxicological significance of these findings is unclear.”*

COMMENT: P 41, Line 24: Insert a ‘period’ after the reference.

RESPONSE: *This comment refers to the missing period in the following sentence in section 2.5 (Cardiovascular – Inhalation): “No organ weight or histopathological changes related to the heart were observed after intermediate inhalation exposure to 2-nitrophenol at concentrations up to 61.5 mg/m3 (Hazleton 1984)” A period was added at the end of the sentence, which now reads: “No organ weight or histopathological changes related to the heart were observed after intermediate inhalation exposure to 2-nitrophenol at concentrations up to 61.5 mg/m3 (Hazleton 1984).”*

COMMENT: P44, Line 12: “suspected target” should be replaced with “critical end points” and this suggestion applies for other places on the report.

RESPONSE: *This comment refers to the following sentence in section 2.7 (Hematological – Inhalation): “Based on a systematic evaluation of the literature, hematological effects are a suspected target of exposure to 4-nitrophenol.” The phrase “suspected target” has been changed to “suspected health effects,” and the sentence now reads: “Based on a systematic evaluation of the literature, hematological effects are considered suspected health effects of exposure to 4-nitrophenol.”*

COMMENT: P45, line 18: “toxicological significance”. It is also informative to add “mechanistic link” or mechanism of toxicity, in this sentence.

RESPONSE: *This comment refers to the following sentence in section 2.7 (Hematological – Inhalation): “As these results did not show a clear dose-response relationship between 4-nitrophenol exposure and increases in methemoglobin, the toxicological significance of the finding is unclear.” The phrase “mechanistic link” was added, and the sentence now reads: “As these results did not show a clear dose-response relationship between 4-nitrophenol exposure and increases in methemoglobin, the mechanistic link and the toxicological significance of the finding are unclear.”*

COMMENT: P46, line 14-15, line 26-27: “No musculoskeletal effects were observed in two studies of inhalation exposure to 2-nitrophenol or 4-nitrophenol”. It may be useful to show exactly what musculoskeletal end points were measured in the study.

RESPONSE: *This comment refers to the following sentences in section 2.8 (Musculoskeletal): "No musculoskeletal effects were observed in two studies of inhalation exposure to 2-nitrophenol or 4-nitrophenol. Hazleton (found no musculoskeletal effects in rats following intermittent exposure to 2-nitrophenol for 4 weeks at concentrations up to 61.5 mg/m^3. Similarly, Hazleton found no musculoskeletal effects of intermediate inhalation exposure to 4-nitrophenol in rats at concentrations up to 30 mg/m^3. "; and "Hazleton found no musculoskeletal effects of gavage administered 4-nitrophenol 7 days/week for 13 weeks at doses up to 140 mg/kg/day." Additional sentences were added to the profile in response to this comment to specify which musculoskeletal components were monitored for changes. Thus, the sentences were updated to read as follows: "No musculoskeletal effects were observed in two studies of inhalation exposure to 2-nitrophenol or 4-nitrophenol. Hazleton found no musculoskeletal effects in rats following intermittent exposure to 2-nitrophenol for 4 weeks at concentrations up to 61.5 mg/m^3. This study monitored for changes in the thoracic spinal cord, skeletal muscle, sternum, femur, and head. Similarly, Hazleton found no musculoskeletal effects of intermediate inhalation exposure to 4-nitrophenol in rats at concentrations up to 30 mg/m^3. Hazleton (1983) monitored for changes in the thoracic spinal cord, skeletal muscle, sternum, and femur." The sentence related to the oral study was updated as follows: "Hazleton found no musculoskeletal effects of gavage administered 4-nitrophenol 7 days/week for 13 weeks at doses up to 140 mg/kg/day. This study monitored for changes in skeletal muscle, the sternum, the femur, and the head."*

COMMENT: P46, line 24: The statement about effects in lower dose and no effects in higher dose is confusing, Please rephrase the sentence "The authors of the study did not speculate as to the reason for the lack of significant effect of 2-nitrophenol on liver weight or liver/brain weight ratio in the higher dose female groups". It can be simply said "The reason for the lack of dose-dependency in the observed hepatic effects was not known".

RESPONSE: *This comment refers to the following sentence in section 2.9 (Hepatic - Inhalation): "The authors of the study did not speculate as to the reason for the lack of significant effect of 2-nitrophenol on liver weight or liver/brain weight ratio in the higher dose female groups." The sentence was revised as requested, and now reads: "The reason for the lack of dose-dependency in the observed hepatic effects was not known."*

COMMENT: P48, line 14: "in Li et al" should be "by Li et al."

RESPONSE: *This comment refers to the following sentence in section 2.9 (Hepatic – Oral): "A second study in Li et al. (2017) observed a widening of the hepatic sinusoid after 3 daily gavage doses of 200 mg/kg 4-nitrophenol, along with further disordering of hepatocytes." The word "in" was changed to "by," and the sentence now reads: "A second study by Li et al. (2017) observed a widening of the hepatic sinusoid after 3 daily gavage doses of 200 mg/kg 4-nitrophenol, along with further disordering of hepatocytes."*

COMMENT: P62, line 29 and 30: "(Shimizu & Yano, 1986))" should be "(Shimizu & Yano, 1986)) ; opening parenthesis missing

RESPONSE: *This comment refers to the following sentence in section 2.19 (Genotoxicity): "One positive result was reported by Shimizu and Yano 1986), who showed that 4-nitrophenol induced DNA damage when tested in B. subtilis by the ret assay. According to Shimizu and Yano 1986), this assay appears to be more sensitive for nitro compounds in general than the standard Ames Test." The reference formats have been fixed, and the sentence now reads: "One positive result was reported by Shimizu and Yano (1986), who showed that 4-nitrophenol induced DNA damage when tested in B. subtilis by the ret assay.*

According to Shimizu and Yano (1986), this assay appears to be more sensitive for nitro compounds in general than the standard Ames Test."

COMMENT: P63, lines 8-9: "found that 4-nitrophenol induced chromosomal aberrations in Chinese hamster ovary cells with activation but not without" This is an incomplete sentence.

RESPONSE: *This comment refers to the following sentence in section 2.19 (Genotoxicity): "found that 4-nitrophenol induced chromosomal aberrations in Chinese hamster ovary cells with activation but not without." A source was added to complete the sentence, which now reads: "NTP (1993) found that 4-nitrophenol induced chromosomal aberrations in Chinese hamster ovary cells with activation but not without."*

COMMENT: P63, line 13: The word 'negative results' would be less confusing than 'negative data'

RESPONSE: *This comment refers to the following sentence in section 2.19 (Genotoxicity): "Taken together, the in vitro and in vivo information, negative data or lack of data, respectively, would suggest that 2-nitrophenol and 4-nitrophenol do not pose a genotoxic threat to humans." The word "data" was changed to "results," and the sentence now reads: "Taken together, the in vitro and in vivo information, negative results or lack of data, respectively, would suggest that 2-nitrophenol and 4-nitrophenol do not pose a genotoxic threat to humans."*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

COMMENT: This section provides adequate discussion of absorption, distribution, metabolism, and excretion of nitrophenols. The available studies are limited and most of the existing studies involved rat models. A summary of salient findings may be presented at the end of each section. Although biological half-life of nitrophenols is presented from a couple of studies, the readers would benefit from a summary of that information. No pharmacokinetic/pharmacodynamic models have been developed for this class of chemicals. Similar no extrapolations could be made from animal to humans for metabolism, due to the existence of limited data.

RESPONSE: *The review suggests a summary of salient findings to be presented at the end of each section. These summaries are present at the beginning of Chapter 3. As such, no changes were made to the profile based on this comment. A sentence was added to the summary related to Excretion regarding the range of observed half-lives for nitrophenols in the literature in response to the review comment related to providing a summary of half-life information. This additional sentence reads: "Observed half-life values for 4-nitrophenol ranged from 0.6 to 5 hours depending on species, exposure pathways, and sex in the animal literature."*

COMMENT: Although there is a subsection on children and vulnerable population, the focus is a bit different from what one would normally expect. For example, exposure in children could be linked to developmental toxicity described in the previous chapter. The discussion should be on whether children could be exposed and if so, are they vulnerable based on the developmental toxicological evidence described in chapter 2. If no inference can be drawn based on the available information, this needs to be explicitly mentioned. Few rodent studies reported effects in F2 and that may be identified here.

RESPONSE: *Section 2.17 (Developmental) shows that there is no evidence in the literature to suggest developmental effects as a result of exposure to nitrophenols. There is one study (Abu-Qare et al. 2000)*

that suggests 4-nitrophenol crosses the placental barrier, but otherwise there is not enough information available in the literature to draw conclusions on whether children are especially vulnerable to health effects from exposure to nitrophenols. The following language has been added to Section 3.2 (Children and Other Populations that are Unusually Susceptible): "Therefore, infants (as well as individuals congenitally deficient in this enzyme), and potentially pregnant women and their fetuses, may represent unusually susceptible subpopulations, as there is some evidence to suggest that 4-nitrophenol crosses the placental barrier (Abu-Qare et al. 2000; . Though generally speaking, more research is needed to determine if children are especially susceptible to the health effects of exposure to nitrophenols."

COMMENT: The subsection on biomarkers is important, but no conclusive statement was made on whether exposures could be assessed through biomarkers. It is important to state firmly that urinalysis can be used to assess nitrophenol exposures. NHANES does report urinary 4-nitrophenol levels, although, as discussed, it could be a metabolite of parathion and several other nitro-aromatics. Adequate discussions have been made on high risk/susceptible populations. There is comprehensive and very useful discussion of the interactive effects of nitrophenols with other substances.

RESPONSE: *Section 3.3 discusses biomarkers of exposure and biomarkers of effect. The text specifies that although 2- and 4-nitrophenol may be able to be used as biomarkers of exposure, their use may be limited to acute exposures only. The text also points out that urinary 4-nitrophenol has been measured in NHANES, but these levels could be due to exposure to 4-nitrophenol or another chemical for which 4-nitrophenol is a metabolite. The sentences in the text that specify these points are as follows: "While the presence of 4-nitrophenol in the urine may be due to exposure to 4-nitrophenol itself, it may also be the result of exposure to other chemicals such as methyl parathion, and nitrobenzene, of which 4-nitrophenol is a metabolite (Barr et al. 2002; EPA 2009; Li and Kannan 2018; Li et al. 2019),, confounding its use as a biomarker of exposure. Due to the rapid excretion of 2- and 4-nitrophenol conjugates in the urine, their use as biomarkers of exposure may be limited to acute exposures only. Based on the current body of literature, it is not known if urinary excretion of 2- or 4-nitrophenol (or their conjugates) can be associated quantitatively with exposure to these chemicals. NHANES data (see Table 5-7) identifies the levels of 4-nitrophenol in urine; however, this could be due to exposure to 4-nitrophenol itself or to a chemical that is metabolized to 4-nitrophenol." No changes were made to the profile based on this comment.*

COMMENT: P65, line 22: It is not sulfuric acid that sulfates molecules. It is inorganic sulfate that contributes to sulfation.

RESPONSE: *This comment refers to the following sentence in section 3.1 (Toxicokinetics): "The resulting metabolites and the parent compounds undergo phase II biotransformation reactions, which include conjugation with glucuronic acid to form glucuronides, sulfuric acid to form sulfates, and for 4-nitrophenol, glutathione to form mercapturic acid derivatives." The phrase "sulfuric acid" was changed to "inorganic sulfates," and the sentence now reads: "The resulting metabolites and the parent compounds undergo phase II biotransformation reactions, which include conjugation with glucuronic acid to form glucuronides, inorganic sulfates to form sulfates, and for 4-nitrophenol, glutathione to form mercapturic acid derivatives."*

COMMENT: P67, line 28: Insert a period at the end of the sentence.

RESPONSE: *This comment refers to the following sentence in section 3.1.2 (Distribution): "4-nitrophenol is minimally distributed in animals after dermal exposure." A period was added at the end of the sentence, which now reads: "4-nitrophenol is minimally distributed in animals after dermal exposure."*

COMMENT: P68, Line 26: "…A pathway figure.." Rephrase as "metabolic pathway" instead of 'pathway'

RESPONSE: *This comment refers to the following sentence in section 3.1.3 (Metabolism): "A pathway figure for the metabolism of 3-nitrophenol has not been identified." The word "metabolic" was added, and the sentence now reads: "A metabolic pathway figure for the metabolism of 3-nitrophenol has not been identified."*

COMMENT: P69, Lines 3-4: this sentence is confusing and needs more clarity. If 70% is glucuronidated, then how 80% is unchanged as nitro group

RESPONSE: *This comment refers to the following sentence in section 3.1.3 (Metabolism): "In these rabbits, conjugation was almost complete with 70% of the dose excreted in urine being in the form of nitrophenol glucuronides (Robinson et al. 1951b). 80% of the nitro group was excreted in urine and unchanged." This information was verified, and "of the nitrophenols" was added, so the now sentence now reads: "In these rabbits, conjugation was almost complete with 70% of the dose excreted in urine being in the form of nitrophenol glucuronides (Robinson et al. 1951b). 80% of the nitro group of the nitrophenols was excreted in urine and unchanged."*

COMMENT: P69, line 9: Not 'sulfuric acid', it is inorganic sulfate.

RESPONSE: *This comment refers to the following sentence in section 3.1.3 (Metabolism): "The two metabolites and the parent compound undergo phase II metabolism through conjugation with glucuronic acid to from glucuronides and sulfuric acid to form sulfates (Robinson et al. 1951ba)." The phrase "sulfuric acid" was changed to "inorganic sulfates," and the sentence now reads: "The two metabolites and the parent compound undergo phase II metabolism through conjugation with glucuronic acid to from glucuronides and inorganic sulfates to form sulfates (Robinson et al. 1951a)."*

COMMENT: P72, line 25: "discusses" should be "discuss"

RESPONSE: *This comment refers to the following sentence in section 3.1.4 (Excretion): "Smith et al. (1988) discusses a decrease in urine volume after exposure to 4-nitrophenol via inhalation, but further details about excretion of the parent compound and the metabolites are not reported (Smith et al. 1988)." The word "discusses" was changed to "discuss," and the sentence now reads: "Smith et al. (1988) discuss a decrease in urine volume after exposure to 4-nitrophenol via inhalation, but further details about excretion of the parent compound and the metabolites are not reported (Smith et al. 1988)."*

COMMENT: P77, line 24: 2-Nitrophenolis should be 2-Nitrophenol is (space missing)

RESPONSE: *This comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "2-Nitrophenolis metabolized to 2-aminphenol, nitroquinone, sulfate conjugates, and glucuronyl conjugate." The word "2-Nitrophenolis" was changed to "2-Nitrophenol is," and the sentence now reads: "2-Nitrophenol is metabolized to 2-aminphenol, nitroquinone, sulfate conjugates, and glucuronyl conjugate."*

COMMENT: P78, line 18, 19: Remove "Tang et al. (2016) demonstrated that", as this reference is cited at the end of the sentence. Start as "Oral exposure….."

RESPONSE: *This comment refers to the following sentence in section 3.3.2 (Biomarkers of Effect): "Tang et al. (2016) demonstrated that oral exposure to 4-nitrophenol altered the expression of cytochrome P450 and other enzymes in the small intestine in male Wistar rats (Tang et al. 2016)." The reference at the start of the sentence was removed, so it now reads: "Oral exposure to 4-nitrophenol*

altered the expression of cytochrome P450 and other enzymes in the small intestine in male Wistar rats (Tang et al. 2016)."

Chapter 4. Chemical and Physical Information

COMMENT: The values and information provided in the chemical and physical properties tables are correct. Adequate information is presented on various forms of the substance. This chapter may be presented as chapter 2, as it introduces the chemical in the report.

P83, line 4: "which could allow global dispersion via precipitation"; substances with high vapor pressure do not necessarily allow for global dispersion via precipitation. Substances with low vapor pressures are less prone to long range atmospheric transportation.

RESPONSE: *Thank you. This information is contained in chapter 4 per ATSDR toxicological profile development guidance.*

The P83 Line 4 comment refers to the following sentence in section 4.2 (Physical and Chemical Properties): "They also have low vapor pressures, which could allow global dispersion via precipitation (Harrison et al. 2005)." This sentence was updated to point out that low vapor pressures indicate the potential for long range atmospheric transport is low. The sentence now reads "They also have low vapor pressures, and therefore potential for long range atmospheric transport is low."

Chapter 5. Potential for Human Exposure

COMMENT: This chapter may be presented as chapter 3 (move forward), after the chapter on physical and chemical information. The chapter is comprehensive and presents all the information available on production, import/export, use, and disposal of nitrophenols. The environmental fate is well described. It is clear that high water solubility dictates the fate and also implies that aquatic environment is the sink for this class of chemicals. These chemicals have relatively low vapor pressure and therefore partitioning of them to air is minimal. Their partitioning to sediment/soil is limited as well. NPL sites contaminated with nitrophenols have been identified clearly. It would have been informative if the contamination levels of water/groundwater in those sites were assessed and if drinking water monitoring is conducted in those locations. Environmental monitoring data are appropriately presented. For sediment/soil data, concentrations should be identified as 'dry weight basis'. The discussion of environmental monitoring data is adequate. Potential human exposure pathways are described appropriately.

RESPONSE: *The chapter headings and order in the profile is defined by ATSDR guidance; thus, no changes can be made to the layout of the profile as it is considered standard across all ATSDR Toxicological Profiles. With respect to the NPL site data, Table 5-4 presents nitrophenols levels in water, soil, and air at NPL sites. Unfortunately, the information contained in the NPL site contamination data does not allow for the presentation of information related to whether or not drinking water monitoring was being conducted for these locations. As such, this information cannot be added to the profile. A footnote was added to Table 5-6 specifying that concentrations in sediment/soil are on a dry weight basis based on this comment. Specifically, the footnote states: "The sediment concentrations are reported on a dry weight basis."*

COMMENT: In a statement on P95, other media, it is stated that 4-nitrophenol would biomagnify from lower to higher tropic levels in both aquatic and terrestrial organisms. This statement needs reevaluation. The fish studies show bioconcentration not biomagnification in the food chain. So please revise as "4-nitrophenol would bioconcentrate slightly, but the evidence for biomagnification is lacking.

RESPONSE: *The change suggested is referring to the following sentence in Section 5.4.1 (Transport and Partitioning): "Based on available BCFs, 4-nitrophenol would biomagnify from lower to higher tropic levels in both aquatic and terrestrial organisms (Loehr & Krishnamoorrthy, 1988)." The suggested change was made, and the updated sentence reads as follows: "Based on available BCFs, 4-nitrophenol would bioconcentrate slightly, but the evidence for biomagnification is lacking (Loehr & Krishnamoorrthy, 1988)."*

COMMENT: Underground injection of 2-nitrophenol at >6000 pounds per year has been reported in one production site. Implications for ground water contamination in that site is not discussed. Considering high water solubility and lack of abiotic transformation in ground water, this raises concern of contamination in local areas and this needs some discussion. Populations living near those sites where nitrophenols are injected into soil are vulnerable for high exposures and that should be identified. Owing to high water solubility of these chemicals, ground water contamination at production sites should be given consideration for exposure assessment studies.

RESPONSE: *This comment is referring to information presented in Table 5-2 at the beginning of Section 5.3 (Releases to the Environment). This table portrays information contained within the Toxics Release Inventory dataset and is meant to be a summary table for chemical releases by state. The data do not contain information about where these underground injections are taking place, as they are predominantly happening off-site. As such, we cannot speculate as to the implications for groundwater contamination near this particular site. No changes were made to the profile based on this comment.*

COMMENT: P87, line 28: semialehyde should be "semialdehyde"

RESPONSE: *This comment refers to the following sentence in section 5.1 (Overview): "Catechol, beta-keto adipic acid, and nitrite have been identified as products of aerobic biodegradation of 2-nitrophenol (Zeyer and Kearney 1984) and 4-nitrocatechol, hydroquinone, gamma-hydroxymuconic semialehyde, and nitrite from 4-nitrophenol (Spain et al. 1979)." The missing 'd' in "semialdehyde" was added, so the sentence now reads: "Catechol, beta-keto adipic acid, and nitrite have been identified as products of aerobic biodegradation of 2-nitrophenol (Zeyer and Kearney 1984) and 4-nitrocatechol, hydroquinone, gamma-hydroxymuconic semialdehyde, and nitrite from 4-nitrophenol (Spain et al. 1979)."*

COMMENT: P88, line 16-17: "Farmworkers have been shown to have significantly higher mean creatinine-adjusted concentrations of urinary 4-nitrophenol than the general population" This statement needs a reference.

RESPONSE: *This comment refers to the following sentence in section 5.1 (Overview): "Farmworkers have been shown to have significantly higher mean creatinine-adjusted concentrations of urinary 4-nitrophenol than the general population." A reference for this statement was added, and the sentence now reads: "Farmworkers have been shown to have significantly higher mean creatinine-adjusted concentrations of urinary 4-nitrophenol than the general population (López-Gálvez et al. 2018)."*

COMMENT: P91, line 4: These releases are summarized in Tables 5-3 and 5-4. The releases are reported in Table 5-2, NOT 5-3 or 5-4.

RESPONSE: *This comment refers to the following sentence in section 5.3 (Releases to the Environment): "Reported amounts of 2- and 4-nitrophenols released to the environment by U.S. facilities are reported in Table 5-3 and 5-4." Tables 5-3 and 5-4 were replaced by the correct table, Table 5-2, so the sentence now reads: "Reported amounts of 2- and 4-nitrophenols released to the environment by U.S. facilities are reported in Table 5-2."*

COMMENT: P92, lines 15 and 18: The releases are reported in Table 5-2, NOT 5-3 or 5-4.

RESPONSE: *This comment refers to the following sentence in section 5.3.1 (Air): "These releases were summarized in Tables 5-3 and 5-4." Tables 5-3 and 5-4 were replaced by the correct table, Table 5-2, so the sentence now reads: "These releases were summarized in Table 5-2."*

COMMENT: P93, lines 5-6: The releases are reported in Table 5-2, NOT 5-4.

RESPONSE: *This comment refers to the following sentence in section 5.3.2 (Water): "These releases were summarized in Table 5-4." Table 5-4 was replaced by the correct table, Table 5-2, so the sentence now reads: "These releases were summarized in Table 5-2."*

COMMENT: P95, lines 19, 22: species names should be italicized.

RESPONSE: *This comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Other Media): "In golden orfe fish (Leuciscus idus melanotus), the whole-body BCF after three days of exposure was 57 (Freitag et al. 1982). With 14C radiolabeled test compound, the mean plateau whole-body 14C BCF for 4-nitrophenol in the fathead minnow (Pimephales oromelas) was 180." The species names were italicized, so the sentence now reads: "In golden orfe fish (Leuciscus idus melanotus), the whole-body BCF after three days of exposure was 57 (Freitag et al. 1982). With 14C radiolabeled test compound, the mean plateau whole-body 14C BCF for 4-nitrophenol in the fathead minnow (Pimephales oromelas) was 180."*

COMMENT: P95,lines 26, 27: "4-nitrophenol would biomagnify from lower to higher tropic levels in both aquatic and terrestrial organisms" This statement needs reevaluation. The fish studies show bioconcentration not biomagnification in the food chain. So please revise as "4-nitrophenol would bioconcentrate slightly, but the evidence for biomagnification is lacking.

RESPONSE: *This comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Other Media): "Based on available BCFs, 4-nitrophenol would biomagnify from lower to higher tropic levels in both aquatic and terrestrial organisms." The sentence was revised as requested, and now reads: "Based on available BCFs, 4-nitrophenol would bioconcentrate slightly, but the evidence for biomagnification is lacking (Loehr and Krishnamoorthy 1988)."*

COMMENT: P103, Table 5-8: State if the sediment concentrations are reported on a dry weight basis. It can be listed in the footnote.

RESPONSE: *This comment refers to Table 5-6 in section 5.5.3 (Sediment and Soil). A footnote was added clarifying that "The sediment concentrations are reported on a dry weight basis."*

COMMENT: P106, line 16: change/convert nmol/L to ug/L.

RESPONSE: *This comment refers to the following sentence in section 5.7 (Populations with Potentially High Exposures): "The mean concentration of urinary 4-nitrophenol was 32 nmol/l." The units were changed, so the sentence now reads: "The mean concentration of urinary 4-nitrophenol was 11.6 µg/L."*

Chapter 6. Adequacy of the Database

COMMENT: The document clearly identified that there is a general lack of literature on the health effects of 2-, 3-, and 4-nitrophenol. Existing toxicological studies are old and there is a need for sub-chronic long term low dose exposures. The data needs are presented in a neutral, and non-judgmental fashion.

P100, lines 20-21: As suggested earlier, there appears to be more serious reproductive effects especially effects on reproductive hormones (Page 59-Page 60). This further indicates the need for studies on endocrine related effects. I would add some statement on this regard in this section. These studies should be conducted systematically with dose response relationships and at exposures levels relevant for human populations.

RESPONSE: *Thank you very much. This comment appears to refer to Section 6.2 (Identification of Data Needs). In response to this comment, additional language was added beneath the "Health Effects" subheading of this Section to specify that reproductive/endocrine effects should be studied further at exposure levels relevant to human populations. The relevant sentences read as follows: "There were a large number of intraperitoneal studies that showed potential reproductive/endocrine effects in both male and female rats and mice, however, there were no studies using routes of exposure considered sufficient for MRL development, such as inhalation, oral, or dermal exposure. A data need exists for the study of reproductive/endocrine effects using these human-relevant routes of exposure at exposure levels relevant for human populations."*

COMMENT: P113, line 19: The environmental release of nitrophenols from other uses such as rubber production, pigment/dye production is not well known and their contribution to human exposure is not known.

RESPONSE: *This comment refers to the Production, Import/Export, Use, Release, and Disposal subsection of section 6.2 (Identification of Data Needs). Under the Release heading, the sentence "There does not appear to be a need for additional data on releases of nitrophenols" has been deleted, and the following sentence has been added: "Additional data is needed on the environmental release of nitrophenols from uses such as rubber production and pigment/dye production to adequately assess their contribution to human exposure."*

COMMENT: P109, line 17: Insert a period at the end of the sentence and the leave a space.

RESPONSE: *This comment refers to the following sentences in section 6.2 (Identification of Data Needs – Acute-Duration MRLs): "Additional studies are needed to characterize health effects for lower-level oral doses of 4-nitrophenol Studies are needed to characterize health effects following acute exposure to 2- and 3-nitrophenol." A period was added at the end of the first sentence: "Additional studies are needed to characterize health effects for lower-level oral doses of 4-nitrophenol. Studies are needed to characterize health effects following acute exposure to 2- and 3-nitrophenol."*

COMMENT: P111, line 21: 'concentrations(Hazleton 1983)", Insert space after 'concentrations'.

RESPONSE: *This comment refers to the following sentence in section 6.2 (Identification of Data Needs – Hematological): "Additionally, an intermediate- duration inhalation study in Sprague-Dawley rats showed no hematological effects, albeit at lower concentrations(Hazleton 1983)." A space was added between "concentrations" and the reference, so the sentence now reads: "Additionally, an intermediate-*

duration inhalation study in Sprague-Dawley rats showed no hematological effects, albeit at lower concentrations (Hazleton 1983)."

COMMENT: P112, line 6: Epidemiology and Human Dosimetry Studies. I would also suggest the need for studies on reproductive effects and hormonal changes.

RESPONSE: *This comment refers to the Epidemiology and Human Dosimetry Studies subheading in section 6.2 (Identification of Data Needs). In response to this comment, reproductive and endocrine studies were added to the list of health outcomes with no information identified in humans. The first sentence in this section now reads: "No information regarding respiratory, ocular, reproductive, endocrine, and hematological effects associated with human exposure to nitrophenols were identified."*

COMMENT: P114, line 14: rephrase "Since the compounds have low vapor pressures, global dispersion via precipitation is possible" as "Since these compounds have low vapor pressures, their potential for long range atmospheric transport is low",

RESPONSE: *This comment refers to the following sentence in section 6.2 (Identification of Data Needs – Environmental Fate): "Since the compounds have low vapor pressures, global dispersion via precipitation is possible." The sentence was revised as requested, and now reads: "Since these compounds have low vapor pressures, their potential for long range atmospheric transport is low."*

Chapter 7. Regulations and Guidelines

COMMENT: This section captures all the available regulatory information for the three nitrophenols. No additional regulatory data were found

RESPONSE: *No revisions were suggested.*

Additional References from Reviewer*

**These are references cited within the reviewer's individual comments. Responses to the reviewer's comments specify the disposition of these references within the toxicological profile.*

Appendices

COMMENT: The appendices are well presented starting from literature search strategy to laying out conditions and criteria for reviews and deriving conclusions. Very informative section.

RESPONSE: *No revisions were suggested.*

Unpublished Studies (If Applicable to Review)

COMMENT: Reviewer 2 comments on "Monsanto. 1990. Range-Finding Teratology Study In Rats. St. Louis, MO." can be found in the attached file named "Reviewer 2 Comments Unpublished Study.pdf".

RESPONSE: *Based on the reviewer comments, the aforementioned study has been included in the profile.*

Annotated Comments on the Profile

COMMENT: They do not depict respiratory or hematological effects and do not support the conclusions. It is important to list respiratory and additional hematological effects on these two figures

RESPONSE*: This comment is found in Section 1.2 (Summary of Health Effects). This comment is referring to the information found in Figure 1-1 entitled "Health Effects Found in Animals Following Inhalation Exposure to 4-Nitrophenol" and Figure 1-2 entitled "Health Effects Found in Animals Following Oral Exposure to 4-Nitrophenol". These figures do indeed depict respiratory and hematological effects that have been observed in studies included in the LSE tables. Figure 1-1 shows increased methemoglobin after acute inhalation exposure to 130 to 340 mg/m^3 of 4-nitrophenol, and Figure 2-2 shows wheezing and dyspnea after intermediate oral exposure to 25 to 70 mg/kg/day of 4-nitrophenol. No changes were made to the profile based on this comment.*

COMMENT: Only inhalation exposure resulted in hematological effect (not oral exposure)

RESPONSE*: This comment refers to the following sentence in Section 1.2 (Summary of Health Effects – Hematological Effects): "Hematological effects were not observed in acute- or intermediate-duration oral studies of 4-nitrophenol in rats (Abu-Qare et al. 2000; Hazleton 1989)." The sentence states that there were not observed in studies where animals were exposed to nitrophenols orally- 'Hematological effects were not observed in acute or intermediate oral studies of 4-nitrophenol in rats (Abu-Qare et al. 2000; Hazleton 1989).' No changes were made to the profile based on this comment.*

COMMENT: A statement on very high doses used in animal studies would further justify inability to drive an MRL

RESPONSE*: This comment is found in Section 1.3 (Minimal Risk Levels (MRLs)). The following statement was added to the profile: "The sensitive endpoints observed in animal studies are at relatively high doses compared to typical human exposures."*

COMMENT: Critical info,

RESPONSE*: No changes were suggested in this comment, and as such, no changes were made to the profile.*

COMMENT: Critical info

RESPONSE*: No changes were suggested in this comment, and as such, no changes were made to the profile.*

COMMENT: This is a critical information, not presented in the figure 2.2 although present in Table 2.1 . I think this is a key information that may be presented in overall summary

RESPONSE*: This comment is related to a sentence in Section 2.4 (Respiratory) that reads: "The 5 mg/m^3 dose is considered the NOAEL for respiratory effects in this study." The reviewer specifies that the 5 mg/m^3 concentration that is considered the NOAEL for respiratory effects from Hazleton (1984) does not show up in Figure 2-2. This was an accidental omission from this figure. The 5 mg/m^3 NOAEL is now present in the updated version of Figure 2-2.*

COMMENT: Critical info

RESPONSE*: No changes were suggested in this comment, and as such, no changes were made to the profile.*

COMMENT: Critical info

RESPONSE*: No changes were suggested in this comment, and as such, no changes were made to the profile.*

COMMENT: is

RESPONSE*: The comment refers to the following sentence in Section 2.4 (Respiratory – Other): "The toxicological significance of these findings are unclear." The "are" has been changed to "is," and the sentence now reads: "The toxicological significance of these findings is unclear."*

COMMENT: critical info.

RESPONSE*: No changes were suggested in this comment, and as such, no changes were made to the profile.*

COMMENT: critical info

RESPONSE*: No changes were suggested in this comment, and as such, no changes were made to the profile.*

COMMENT: NTP

RESPONSE*: This comment is found in Section 2.18 (Cancer) and relates to the following sentence: "The National Toxicology Program of the U.S. Department of Health and Human Services has not classified the nitrophenols with regard to their human carcinogenicity." In response to this comment, the abbreviation was added to this sentence. The sentence now reads: "The National Toxicology Program of the U.S. Department of Health and Human Services (NTP) has not classified the nitrophenols with regard to their human carcinogenicity."*

COMMENT: Opening parenthesis missing

RESPONSE*: This comment refers to the following sentences in section 2.19 (Genotoxicity): "One positive result was reported by Shimizu and Yano 1986), who showed that 4-nitrophenol induced DNA damage when tested in B. subtilis by the ret assay. According to Shimizu and Yano 1986), this assay appears to be more sensitive for nitro compounds in general than the standard Ames Test. Weaker genotoxic effects were reported in two studies (Adler et al. 1976; Garrett and Lewtas 1983)." The missing parenthesis has been added: "One positive result was reported by Shimizu and Yano (1986), who showed that 4-nitrophenol induced DNA damage when tested in B. subtilis by the ret assay. According to Shimizu and Yano (1986), this assay appears to be more sensitive for nitro compounds in general than the standard Ames Test. Weaker genotoxic effects were reported in two studies (Adler et al. 1976; Garrett and Lewtas 1983)."*

COMMENT: Incomplete sentence

RESPONSE*: This comment refers to the following sentence in section 2.19 (Genotoxicity): "found that 4-nitrophenol induced chromosomal aberrations in Chinese hamster ovary cells with activation but not without." A reference was added to the beginning of the sentence: "NTP (1993) found that 4-nitrophenol induced chromosomal aberrations in Chinese hamster ovary cells with activation but not without."*

COMMENT: 'Results' would be better than 'data'

RESPONSE*: This comment is found in section 2.19 (Genotoxicity) – P63 line 13. The sentence is "Taken together, the in vitro and in vivo information, negative data or lack of data, respectively, would suggest that 2-nitrophenol and 4-nitrophenol do not pose a genotoxic threat to humans." Change was made as requested and now reads: "Taken together, the in vitro and in vivo information, negative results or lack of data, respectively, would suggest that 2-nitrophenol and 4-nitrophenol do not pose a genotoxic threat to humans."*

COMMENT: Inorganic sulfate, not sulfuric acid

RESPONSE*: This comment refers to the following sentence in section 3.1 (Toxicokinetics):"The resulting metabolites and the parent compounds undergo phase II biotransformation reactions, which include conjugation with glucuronic acid to from glucuronides, sulfuric acid to form sulfates, and for 4-nitrophenol, glutathione to form mercapturic acid derivatives." The phrase "sulfuric acid" was changed to "inorganic sulfate," and the sentence now reads: "The resulting metabolites and the parent compounds undergo phase II biotransformation reactions, which include conjugation with glucuronic acid to from glucuronides, inorganic sulfates to form sulfates, and for 4-nitrophenol, glutathione to form mercapturic acid derivatives."*

COMMENT: Inserted period

RESPONSE*: Change accepted.*

COMMENT: Insert "metabolic pathway" instead of pathway

RESPONSE*: The comment refers to the following sentence in section 3.1.3 (Metabolism): "A pathway figure for the metabolism of 3-nitrophenol has not been identified." The word "metabolic" was added, and the sentence now reads: "A metabolic pathway figure for the metabolism of 3-nitrophenol has not been identified."*

COMMENT: Confusing. If 70% is glucuronidated, then how 80% is unchanged as nitro group

RESPONSE*: The comment refers to the following sentence in section 3.1.3 (Metabolism): "In these rabbits, conjugation was almost complete with 70% of the dose excreted in urine being in the form of nitrophenol glucuronides (Robinson et al. 1951b). 80% of the nitro group was excreted in urine and unchanged." This information was verified, and "of the nitrophenols" was added, so the now sentence now reads: "In these rabbits, conjugation was almost complete with 70% of the dose excreted in urine being in the form of nitrophenol glucuronides (Robinson et al. 1951b). 80% of the nitro group of the nitrophenols was excreted in urine and unchanged."*

COMMENT: Not sulfuric acid, it is inorganic sulfate

RESPONSE*: The comment refers to the following sentence in section 3.1.3 (Metabolism): "The two metabolites and the parent compound undergo phase II metabolism through conjugation with glucuronic acid to from glucuronides and sulfuric acid to form sulfates (Robinson et al. 1951ba)." The phrase "sulfuric acid" was changed to "inorganic sulfates," and the sentence now reads: "The two metabolites and the parent compound undergo phase II metabolism through conjugation with glucuronic acid to from glucuronides and inorganic sulfates to form sulfates (Robinson et al. 1951ba)."*

COMMENT: Reference??

RESPONSE*: The comment refers to the following sentence in section 5.1 (Overview): "Farmworkers have been shown to have significantly higher mean creatinine-adjusted concentrations of urinary 4-nitrophenol than the general population." A reference for this statement was added, and the sentence now reads: "Farmworkers have been shown to have significantly higher mean creatinine-adjusted concentrations of urinary 4-nitrophenol than the general population (López-Gálvez et al. 2018)."*

COMMENT: italicize

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Other Media): "In golden orfe fish (Leuciscus idus melanotus), the whole-body BCF after three days of exposure was 57 (Freitag et al. 1982)." The species name was italicized, so the sentence now reads: "In golden orfe fish (Leuciscus idus melanotus), the whole-body BCF after three days of exposure was 57 (Freitag et al. 1982)."*

COMMENT: italicize

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Other Media): "With 14C radiolabeled test compound, the mean plateau whole-body 14C BCF for 4-nitrophenol in the fathead minnow (Pimephales oromelas) was 180." The species name was italicized, so the sentence now reads: "With 14C radiolabeled test compound, the mean plateau whole-body 14C BCF for 4-nitrophenol in the fathead minnow (Pimephales oromelas) was 180."*

COMMENT: spelling. One 'r'

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Other Media): "Based on available BCFs, 4-nitrophenol would bioconcentrate slightly, but the evidence for biomagnification is lacking (Loehr and Krishnamoorrthy 1988)." The extra 'r' in Krishnamoorthy was removed, and the sentence now reads: "Based on available BCFs, 4-nitrophenol would bioconcentrate slightly, but the evidence for biomagnification is lacking (Loehr and Krishnamoorthy 1988)."*

COMMENT: change to ug/L

RESPONSE*: The comment refers to the following sentence in section 5.7 (Populations with Potentially High Exposures): "The mean concentration of urinary 4-nitrophenol was 32 nmol/l." The units were changed, so the sentence now reads: "The mean concentration of urinary 4-nitrophenol was 11.6 µg/L."*

COMMENT: insert space

RESPONSE*: The comment refers to the following sentences in section 6.2 (Identification of Data Needs – Acute-Duration MRLs): "Additional studies are needed to characterize health effects for lower-level oral doses of 4-nitrophenol Studies are needed to characterize health effects following acute exposure to 2- and 3-nitrophenol." A period was added at the end of the first sentence, followed by a space: "Additional studies are needed to characterize health effects for lower-level oral doses of 4-nitrophenol. Studies are needed to characterize health effects following acute exposure to 2- and 3-nitrophenol."*

COMMENT: rephrase, as mentioned earlier

RESPONSE*: The P114 Line 14 comment refers to the following sentence in section 6.2 (Identification of Data Needs – Environmental Fate): "Since the compounds have low vapor pressures, global dispersion via precipitation is possible." The sentence was revised as requested, and now reads: "Since these compounds have low vapor pressures, their potential for long range atmospheric transport is low."*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

Additional general comments provided by Reviewer #2 outside of charge questions:

COMMENT:

i) In a general manner, I am used to present exposure first and health effect associated to a corresponding level of exposure in a second step. I imagine that the ATSDR report have fixed template, so I do not expect that the authors change the order of the parts. However, it is currently difficult to interpret whether levels of exposure used in the different animal studies are realistic or not compared to human exposures. Maybe the authors might use the data presented in Chapter 5 to briefly discuss this point in Chapter 1 or 2.

ii) In the main effect presented in Chapter 1and 6, the authors only focused on hematological, respiratory and ocular effects. The authors might consider adding dermal to suspected effects, since animal studies are quite concordant.

iii) For oral acute exposure to 4-nitrophenol, there enough data to assess adverse health effects. However, since the NOAEL was not available (effect still observed at 200mg/kg/day, the lowest doses), no MRL was derived. Why don't set a (temporary) MRL based on 200mg/kg/day? I assume that limiting exposure at this threshold would be better that nothing…

iv) Some studies presented in the "reproductive" section, Chapter 2, are suggesting estrogenic activities for 4-nitrophenol. Despite these data are limited, the probability of an endocrine disrupting property of 4-nitrophenol should be debated, and included in the Chapter 6.2 (data needed).

v) Since fetal hemoglobin is more likely to turn into methemoglobin, fetus might be more sensitive to nitrophenols exposure. Discussion about the about le ability of nitrophenols to cross the placental barrier should be included to the document, or included in the chapter 6.2.

RESPONSE:

i) The ATSDR Toxicological Profiles do have a fixed template. Information about exposure to the chemicals of interest is presented in Chapter 5, whereas health effect information is presented in Chapter 2. A sentence was added to Section 1.3 that reads: "The sensitive endpoints observed in animal studies are at dose levels that are relatively high compared to those that may be experienced by human populations."

ii) These lists of hazard identification conclusions only focused on those health outcomes specifically analyzed in the systematic reviews performed for Appendix C. Because dermal effects were not analyzed using systematic review principles in Appendix C, we have not added dermal effects to the suspected effects.

iii) It is ATSDR policy to not derive an MRL at a dose level where a serious effect has been observed. Since there are no other adequately conducted studies with observed health effects at acute oral doses of 4-nitrophenol below 200 mg/kg/day, we are unable to derive an acute oral MRL. No changes were made to the profile based on this comment.

iv) All studies presented in Section 2.17 (Reproductive) that contained endocrine-related information were kept in the Reproductive section, but were also added to Section 2.13 (Endocrine), as the results were relevant to both sections of the profile. Regarding the probability of an endocrine disrupting property of 4-nitrophenol, we have included the following sentence in Section 2.13: "Given the lack of corroborating evidence of these observed endocrine outcomes in studies using the oral, inhalation, and dermal routes of

exposure, these results should be treated with caution regarding making generalizations about the effects of 4-nitrophenol on endocrine toxicity." Additionally, the following language is now included in Section 6.2 (Identification of Data Needs): "There were a large number of intraperitoneal studies that showed potential reproductive/endocrine effects in both male and female rats and mice, however, there were no studies using routes of exposure considered relevant to MRL development, such as inhalation, oral, or dermal exposure. A data need exists for the study of reproductive/endocrine effects using these human-relevant routes of exposure at exposure levels relevant for human populations."

v) *In response to this comment, a sentence in Section 3.2 (Children and Other Populations that are Unusually Susceptible) was revised to mention fetuses being sensitive to exposure to nitrophenols. The original sentence read as follows: "Therefore, infants (as well as individuals congenitally deficient in this enzyme) may represent unusually susceptible subpopulations ((Naoum, 2012))." This sentence was revised to read as follows: "Therefore, infants (as well as individuals congenitally deficient in this enzyme), and potentially pregnant women and their fetuses, may represent unusually susceptible subpopulations, as there is some evidence to suggest that 4-nitrophenol crosses the placental barrier (Abu-Qare et al. 2000; (Naoum, 2012)." Additionally, a sentence was added was Section 3.1.2 (Distribution) that reads as follows: "Abu-Qare et al. (2000) also observed distribution of 4-nitrophenol into the tissue of the fetus, suggesting 4-nitrophenol crosses the placental barrier after oral exposure in rats, though no fetal toxic effects were observed due to this exposure to 4-nitrophenol."*

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: Not applicable, no human literature available.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: It is not clear whether levels of exposure used in animal studies are realistic or not compared to human exposures (environmental or occupational). The authors should discuss this point in the Chapter 1 or 2.

RESPONSE: *A sentence was added to Chapter 1 explaining that doses administered in animal studies are expected to be higher than doses experienced by humans. This additional sentence reads: "The sensitive endpoints observed in animal studies are at dose levels that are relatively high compared to those that may be experienced by human populations."*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain

COMMENT: Yes, in general. Some specific comments were added in the text to help interpretation.

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT: Yes for all but one. There enough data to assess adverse health effects for oral acute exposure to 4-nitrophenol. I assume it would be better to set up a MRL based on 200mg/kg/day, than not deriving MRL, even in absence of NOAEL.

RESPONSE: *It is ATSDR policy to not derive an MRL at a dose level where a serious effect has been observed. Since there are no other adequately conducted studies with observed health effects at acute oral doses of 4-nitrophenol below 200 mg/kg/day, we are unable to derive an acute oral MRL. No changes were made to the profile based on this comment.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT: Not applicable (no MRL derivated)

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: No other commentary.

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: No other commentary.

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Not applicable, no human literature available.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Some studies present severe methodological limitations, but these limitations were pointed out by the authors.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Yes, to my knowledge

RESPONSE: *No revisions were suggested.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT: Yes. However, the chance of non-linear relationship might be addressed in the text, especially when the same non-linear pattern is observed in distinct studies (instead of suggesting only a possible false positive – e.g. page 3).

RESPONSE: *Language was added to the text suggesting the possibility that compensatory mechanisms could be responsible for the mitigation of the health effects at higher doses. The previous language in Section 1.2 that read "An intermediate inhalation study of 4-nitrophenol observed a significant increase in methemoglobin in rats at lower concentration groups, but this same result was not observed at higher concentrations, indicating that it might not be a true effect (Hazleton 1983)." has been updated to the following: "An intermediate-duration inhalation study of 4-nitrophenol observed a significant increase in methemoglobin in rats at lower concentration groups, but this same result was not observed at higher concentrations, indicating that it might not be a true effect (Hazleton 1983). It is possible, however, that there are compensatory mechanisms involved when the animals are exposed to the higher doses of nitrophenols, thus mitigating the health effects observed at lower doses."*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: I agree.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: Some additional mechanism of action might be found using toxicogenomic approach. Using the Comparative Toxicogenomic Database, it is possible identify genes associations to specific compounds and disease associated to these genes. As an example, some direct evidence suggesting action on the liver injury are reported for 4-nitrophenol (http://ctdbase.org/detail.go?type=chem&acc=C024836&view=disease).

RESPONSE: *The information related to metabolism and the CYP genes that regulate metabolism is included in Chapter 3 in Section 3.1.3 (Metabolism). The following text is present in the ToxProfile* "*Phase I reactions mediated by cytochrome P450 include oxidation to form 4-nitrocatechol and reduction*

to yield 4-aminophenol (Machida, Morita, Hayashi, & Awazu, 1982). The two polar metabolites and the parent compound undergo phase II biotransformation reaction which includes conjugation with glucuronic acid to from glucuronides, sulfuric acid to form sulfates, and glutathione to form mercapturic acid derivatives (Machida et al., 1982). 4-Nitrophenol hydroxylation to 4-nitrocatechol is mediated by the CYP2E1 enzyme (Abu-Qare, Brownie, & Abou-Donia, 2000).". While gene interactions are not mentioned in the ToxProfile, relevant alterations in health effects are discussed in the profile. Additional gene interactions that are discussed in the Comparative Toxicogenomic Database use either in vitro or animal models that are not considered relevant for developing the ToxProfiles as per the profile development guidance provided by ATSDR. Only studies that examine changes in mRNA expression which also discuss a health effect as a result of that change in expression are included in the profile. For example, CASP3 expression is increased after exposure to 4- nitrophenol in a study by Mi et al. (2013), and is discussed in the profile in Section 2.16(Reproductive) as exposure to 4-nitrophenol caused reproductive deficits in male mice, which is likely a result of change in the gene expression of CASP3. While changes in gene expression of CASP3 are not explicitly discussed in the profile, relevant health effects that are caused by 4-nitrophenol exposure are detailed in Chapter 2 in corresponding sections. As such, no changes were made to the profile based on this comment.

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: Only two remarks:

- "Endocrine" part: two studies currently presented in the "Reproduction" part suggested estrogenic activity of 4-nitrophenol when administrated subcutaneously. These should be replaced here, the probability of an endocrine disrupting property of 4-nitrophenol should be debated.
- "Reproductive" part: the authors disregards positive results observed in five recent studies using subcutaneous administration, since nothing was observed in studies based on oral / inhalation / dermal absorption. At least, the authors should discuss methodological or mechanistic differences susceptible to explain difference in findings.

RESPONSE: *With respect to the first remark, all studies presented in Section 2.17 (Reproductive) that contained endocrine-related information were kept in the Reproductive section, but were also added to Section 2.13 (Endocrine), as the results were relevant to both sections of the profile. Regarding the probability of an endocrine disrupting property of 4-nitrophenol, we have included the following sentence in Section 2.13: "Given the lack of corroborating evidence of these observed endocrine outcomes in studies using the oral, inhalation, and dermal routes of exposure, these results should be treated with caution regarding making generalizations about the effects of 4-nitrophenol on endocrine toxicity."*

With respect to the second remark, these recent intraperitoneal injection studies were not disregarded in the profile. The sentence that suggests these results to be treated with caution regarding making generalizations about the effects of 4-nitrophenol on reproductive or endocrine toxicity was included in the profile because these studies used a route of exposure that is generally only considered as supporting evidence by ATSDR to other information extracted from inhalation, oral, dermal studies. Thus, although these studies provide some evidence that 4-nitrophenol could cause reproductive or endocrine effects after intraperitoneal injections, it is unclear whether these effects would persist if exposure were to occur via the inhalation, oral, or dermal route. As such, no changes were made to the profile based on this portion of the reviewer's comment.

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Yes, only some minor comments in the text.

RESPONSE: *Individual comments made by the reviewer in Chapter 3 are addressed in the "Annotated Comments" section.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT: Not applicable (models unavailable).

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: Not applicable (models unavailable).

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: Not to my knowledge.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: This point was discussed. I agree with the choice of population. Possible impact on fetus should be mentioned, since fetal hemoglobin is more likely to turn into methemoglobin (only possible effect on the newborn was mentioned, for the same reason).

RESPONSE: *No revisions were suggested.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT: There are no specific biomarkers identified. Compounds themselves might be measured in urine or hair, but since nitrophenols can be degradation product to other compounds as well, these are not specific.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT: Not to my knowledge.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: No interaction identified.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT: Yes, there are basic data on boiling point, vapor pressure… etc.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: Substances were difficult to trace from their point of release to the receptor population, since nitrophenols might be used in multiple situation and might derived from compounds extensively used in the environment.

Also, it is difficult to drawn conclusion based on the raw extent of occurrence at NPL site: the true number of contaminated hazardous site per state is not known since all hazardous sites are not reported. In this context, proportion of contaminated among the reported NPL would be more informative.

RESPONSE: *No studies were located that make it possible to definitively conclude the source or compound associated with nitrophenols exposures in the general population or in populations with potentially high exposures.*

The NPL data does not report the number of sites evaluated for nitrophenols. While it may be more informative to report the proportion of contaminated sites, it is not possible to calculate the proportion with the data and Figure 5-1 is a standard figure per ATSDR Profile Development Guidance, so no changes were made.

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes / no other relevant information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes to all / no other relevant information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: Population and specific occupations likely to be more exposure to nitrophenols might be more detailed (the authors only told about "manufacture, use of nitrophenol and application of pesticides"). Also, I assume that population leaving near specific industries or some other occupations (e.g. mechanician, due to engine exhaust) might be more exposed.

RESPONSE: *Aside from farmworkers described in section 5.7 (Populations with Potentially High Exposures), studies examining exposure in workers of specific occupations were not identified. Section 5.7 also discusses potentially high exposures among populations living near landfill sites and agricultural areas. The following sentence has also been added to the first paragraph in section 5.7 to address high exposure due to engine exhaust: "Since nitrophenols are released from car exhaust, potentially high exposures could also occur in populations living near heavy traffic or people who work with or spend time around idling gas powered or diesel powered motor vehicles."*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT: Toxicogenomic data might be used to identify biological mechanism for the different health effect identified. See my previous comment (chapter 2, question 10).

RESPONSE: *The gene interactions that are discussed in the Comparative Toxicogenomic Database use either in vitro or animal models that are not considered relevant for developing the ToxProfiles as per the profile development guidance provided by ATSDR. Other relationships that are reported in this database are already included in the profile. Thus, no changes were made to the profile based on this comment.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT: I would add several points in the data needs:

- To explore possible endocrine disruptor mechanisms
- To explore a possible passage through the placental barrier
- To study more in depth a possible influence of nitrophenols on reproductive effects, due to the inconsistencies between studies using subcutaneous injections, compare to more classical oral/inhalation/dermal exposures.

RESPONSE: *Each of these points have been added to Section 6.2 (Identification of Data Needs). Regarding the first and third points above, the following sentences are contained within Section 6.2 under the "Health Effects" subheading: "There were a large number of intraperitoneal studies that showed potential reproductive/endocrine effects in both male and female rats and mice, however, there were no studies using routes of exposure considered sufficient for MRL derivation, such as inhalation, oral, or dermal exposure. A data need exists for the study of reproductive/endocrine effects using these human-relevant routes of exposure at exposure levels relevant for human populations." Regarding the second point above, the following sentence was added to Section 6.2 under the "Absorption, Distribution,*

Metabolism, and Excretion" subheading: "A specific data need exists for further information regarding the possible distribution of 4-nitrophenol through the placental barrier, as fetal hemoglobin might be more sensitive to the effects of 4-nitrophenol."

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT: I have very limited expertise on the points. Not to my knowledge.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: I have very limited expertise on the points. Not to my knowledge.

RESPONSE: *No revisions were suggested.*

Additional References from Reviewer*

**These are references cited within the reviewer's individual comments. Responses to the reviewer's comments specify the disposition of these references within the toxicological profile.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT:

RESPONSE: *No revisions were suggested.*

Unpublished Studies (If Applicable to Review)

COMMENT: Reviewer 3 comments on "Monsanto. 1990. Range-Finding Teratology Study In Rats. St. Louis, MO." can be found in the attached file named "Reviewer 3 Comments Unpublished Study.doc".

RESPONSE: *Based on the reviewer comments, the aforementioned study has been included in the profile.*

Annotated Comments on the Profile

COMMENT: Fungicide is included in the pesticide definition (as well as lumber preservative, depending on the pesticide definition)

" (…) pesticides, including fungicides and lumber preservatives"?

RESPONSE*: This comment refers to the following sentence in section 1.1 (Overview and U.S. Exposures): "Mononitrophenol isomers are primarily used as intermediates to produce dyes, pigments, pharmaceuticals, rubber chemicals, lumber preservatives, photographic chemicals, pesticides, and fungicides." The sentence was revised as requested and now reads: "Mononitrophenol isomers are primarily used as intermediates to produce dyes, pigments, pharmaceuticals, rubber chemicals, photographic chemicals, pesticides, including fungicides and lumber preservatives."*

COMMENT: There is no reference in this paragraph.

RESPONSE*: The comment refers to the following paragraph in section 1.1 (Overview and U.S. Exposures): "The general population may be exposed to very small amounts of nitrophenols through the inhalation of ambient air. Small amounts of the three substances have been found in the air, water, and soil. Nitrophenols are formed in vehicular exhausts as a result of the thermal reaction of fuel with oxides of nitrogen." An additional sentence was added immediately following this sentence with references that suggest nitrophenols are formed in vehicular exhausts. This added sentence reads as follows: "The nitrophenols are released from exhausts of both gasoline- and diesel-powered vehicles (Inomata, Fushimi, Sato, Fujitani, & Yamada, 2015; Inomata, Yamada, & Tanimoto, 2016; Lu et al., 2019; Nojima, Kawaguchi, Ohya, Kanno, & Hirobe, 1983; Rubio, Bustamante, & Vásquez P, 2019)."*

COMMENT: This sentence is confusing. It is not clear if there is no study focusing on these chemicals, or if these studies exist but retrieved no values >LOD.

RESPONSE*: The comment refers to the following paragraph in section 1.1 (Overview and U.S. Exposures): "Nitrophenols have not been detected in drinking water and foods." The sentence immediately following this sentence states that the absence of detection of nitrophenols in food is either because of a lack of effort directed at monitoring the compounds or because they are present at undetectable levels in food. There is one study mentioned in Section 5.5.4 that found spinach sprayed with parathion did not have higher levels of 4-nitrophenol than non-sprayed spinach, however this was the only study identified that investigated levels of nitrophenols in foods. No changes were made the profile based on this comment.*

COMMENT: …and hair (see Béranger et al. 2018)?

***RESPONSE**: This comment refers to the following sentence in section 1.1 (Overview and U.S. Exposures): "4-Nitrophenol has been detected in human urine." The recommended addition was included and the sentence now reads: "4-Nitrophenol has been detected in human urine and hair (Béranger et al. 2018)."*

COMMENT: Confusing:

There is actually some epidemiological data suggesting associations between PNP and health effect, but it is not possible to distinguish exposure to PNP itself or to its parent compounds.

***RESPONSE**: This comment refers to the following sentence in Section 1.2 (Summary of Health Effects): "No human studies were identified in the literature." A clarification was made to this sentence by specifying the lack of epidemiologic studies specifically focusing on isolated exposure to nitrophenols and associated health effects. The updated sentence reads, "No human studies that focused specifically on isolated exposure to nitrophenols were identified in the literature."*

COMMENT: Adding dermal effects, since animal studies are quite concordant?

***RESPONSE**: This comment is found in section 1.2 (Summary of Health Effects). This list of hazard identification conclusions only focused on those health outcomes specifically analyzed in the systematic reviews performed for Appendix C. Because dermal effects were not analyzed using systematic review principles in Appendix C, we have removed the added sentence regarding dermal effects.*

COMMENT: Non-linear effect are also possible… (e.g. if compensation mechanism are involved at higher doses…).

This should be evoked, especially since another study (Hazelton 1984) observed the same phenomenon.

***RESPONSE**: Included the following language in the text: "An intermediate-duration inhalation study of 4-nitrophenol observed a significant increase in methemoglobin in rats at lower concentration groups, but this same result was not observed at higher concentrations, indicating that it might not be a true effect (Hazleton 1983). It is possible, however, that there are compensatory mechanisms involved when the animals are exposed to the higher doses of nitrophenols, thus mitigating the health effects observed at lower doses." "*

COMMENT: The terms "not classifiable" was used Page 2, line 16. Is it possible to homogenize?

***RESPONSE**: The comment refers to the following sentence in section 1.2 (Summary of Health Effects – Ocular Effects): "Experimental animal studies provide low evidence of an association between 4-nitrophenol exposure and adverse ocular effects, particularly after inhalation and dermal exposure." The term "not classifiable" was used to summarize the entire literature (both animal and human studies) on 4-nitrophenol. This term is used to classify the literature when there is low evidence from animal studies and an absence of human studies, as is the case with 4-nitrophenol. The first sentence in this paragraph states there is an absence of human studies investigating ocular effects after 4-nitrophenol exposure, and this sentence specifies there is low evidence of an association between 4-nitrophenol exposure and adverse ocular effects. Taken together, the systematic review conclusion of "not classifiable" is appropriate, but for animal experimental studies in particular, they should be described as "low evidence". No changes were made to the profile based on this comment.*

COMMENT: I suggest presenting the bars by decreasing order.

RESPONSE*: The comment refers to Figure 2-1 in section 2.1 (Introduction). The order of these endpoints are based on the ATSDR guidelines. They appear in the same order throughout the document. No changes were made to the profile based on this comment.*

COMMENT: Adult?

RESPONSE*: This comment refers to the following sentence found in section 2.2 (Death – Inhalation): "Hazleton (1984) reported no mortality effects in Sprague-Dawley rats after intermittent intermediate-duration inhalation exposure to 2-nitrophenol for 4 weeks at measured concentrations up to 61.5 mg/m^3." The age of the rats was not specified in the study. No changes were made to the profile based on this comment.*

COMMENT: Specify h/day and day/week?

RESPONSE*: : This comment refers to the following sentence found in section 2.2 (Death – Inhalation): "Hazleton (1984) reported no mortality effects in Sprague-Dawley rats after intermittent intermediate-duration inhalation exposure to 2-nitrophenol for 4 weeks at measured concentrations up to 61.5 mg/m^3." The ATSDR profile development guidance specifies that any exposure less than continuous be referred to as "intermittent", and not specifically with the hours/day and days/week. However, because NOAELs for "Death" are not to be reported in the LSE table, we have added this exposure duration information as requested. The revised sentence now reads: "Hazleton (1984) reported no mortality effects in Sprague-Dawley rats after intermittent intermediate-duration inhalation exposure to 2-nitrophenol for 4 weeks, 5 days/week, 6 hours/day at measured concentrations up to 61.5 mg/m^3."*

COMMENT: This formulation in confusing: It is not clear if there is no study focusing on this point, or if these studies exist but retrieved no significant results.

RESPONSE*: This comment refers to the following sentence found in section 2.2 (Death – Inhalation): "There are no available studies showing that inhalation exposure to 4-nitrophenol in animals produces mortality." This is a summary sentence that describes the studies in the next paragraph. These two paragraphs have been combined to clarify. This sentence has also been edited for clarity and now reads: "None of the available animal studies show that inhalation exposure to 4-nitrophenol produces mortality."*

COMMENT: In Annex B, the authors stated that they included only article >1990 (see my comment bellow)

RESPONSE*: This comment refers to the following sentence found in section 2.2 (Death – Oral): "Vernot et al. (1977) estimated LD_{50} values for 2-, 3-, and 4-nitrophenol in rats and mice based on a single oral dose of the chemical." Only studies published after the previous version of this toxicological profile were included in the updated literature search that was described in Appendix B. All studies that were*

included in the previous version of the profile were also included where appropriate. No changes were made to the profile based on this comment.

COMMENT: Unformatted reference.

***RESPONSE**: The comment refers to the following sentence in section 2.2 (Death – Oral): "Death was observed in 4 of 5 male Sprague-Dawley rats after a single gavage dose of 268 mg/kg, and in 3 of 5 Sprague-Dawley female rats after a single gavage dose of 171 mg/kg (Branch and Stout 1983b)." The formatted reference was added in.*

COMMENT: This paragraph is difficult to follow. I suggest presenting doses by croissant order, with the ratio of animal death:

i.e.: 100mg/kg (X death / X animals), 230 mg/kg (X/X), (…), 1000mg/kg (10/12).

***RESPONSE**: The comment refers to the following paragraph in section 2.2 (Death – Oral): "Intermediate administration of 4-nitrophenol has also caused death …" The doses are presented in increasing order of dose for each individual study and are presented in the order of increasing duration. The first study was an 18-day study at doses of 230 mg/kg/day and 320 mg/kg/day. The second study was a 28-day study with doses of 160 mg/kg/day, 400 mg/kg/day, and 1,000 mg/kg/day. The third study was from a different publication and was a 13-week study.*

COMMENT: Please provide more information on the multigenerational study: was the F0 mice exposed at conception and during breastfeeding, or only during pregnancy? Was F1exposures starting a birth?

If all the multigenerational studies presented in the report fit a standardize approach, it might be useful to define more in depth the protocol at the beginning of the section (period covered for F0 and F1 generation, duration of follow up…).

***RESPONSE**: This comment refers to a few sentences in Section 2.2 (Death): "A multigenerational study of 4-nitrophenol in rats did not produce evidence of mortality after dermal daily doses of 250 mg/kg/day for 5 days/week for 20 weeks in the F0 generation, nor following exposure 5 days/ week for 24 weeks in the F1 generation. The dosing of the F1 generation also did not produce any subsequent early mortality in the F2 generation of rats (Angerhofer, 1985)." Further details of the study were added to this description of the Angerhofer (1985) study. These sentences now read as the following: "A multigenerational study of 4-nitrophenol in rats did not produce evidence of mortality after dermal daily doses of 250 mg/kg/day for 5 days/week for 20 weeks in the F0 generation, nor following exposure 5 days/ week for 24 weeks in the F1 generation. The dosing of the F1 generation also did not produce any subsequent early mortality in the F2 generation of rats (Angerhofer, 1985). In this multigenerational study, the original rats purchased for the study were delineated as the F0 generation, and dosing occurred both prior to the mating of the F0 generation, as well as throughout the breeding, gestation, and lactation periods. The F1 generation was weaned approximately 3 weeks after birth, and then were subsequently dosed in the same manner as the F0 generation. The F2 generation was not dosed other than the exposure the rats received in gestation and through the mothers' lactation (Angerhofer 1985)."*

COMMENT: Is it possible to provide the % of body weight?

RESPONSE*: This comment refers to the following sentence in Section 2.3 (Body Weight): "Smith et al. ((1988)) found a suppression of weight gain in an acute study of intermittent 4-nitrophenol inhalation exposure in Albino rats for 2 weeks at levels as low as 294 mg/m^3; however, the study reports no additional quantitative information about the weight gain suppression." Unfortunately, as the sentence states, no additional quantitative information about the weight gain suppression was reported beyond what was qualitatively described in this sentence. As such, no changes were made to the profile based on this comment.*

COMMENT: These results differs from acute exposures at lower dose (paragraph above)… interspecies variations?

RESPONSE*: This comment refers to the following sentence in Section 2.3 (Body Weight): "Intermediate duration exposure to 4-nitrophenol showed no body weight effects following daily doses by gavage of up to 400 mg/kg/day in Sprague-Dawley rats for 18 days, 28 days, or 13 weeks (Hazleton, 1989); (Koizumi et al., 2001)." The literature found mixed results for body weight effects even for animals in the same species. It is possible there are interspecies variations, but because some results from the same species also showed differing results, it would be difficult to make this conclusion. Thus, no changes were made to the profile based on this comment.*

COMMENT: For non-statistically significant results, presenting the number of mice would help to interpret possible lack of statistical power.

RESPONSE*: This comment refers to the following sentence in Section 2.3 (Body Weight – Dermal): "A chronic duration, 78 week, dermal mouse study (Swiss-Webster mice, 3 day per week) of 4-nitrophenol showed no significant body weight effects at doses up to 160 mg/kg (NTP 1993). Based on ATSDR guidance, this information is presented in the LSE tables. In this case, this information is presented in Table 2-2.*

COMMENT: Formulation is confusing. PNP might target a biological tissue, but nor an "exposure" to PNP.

RESPONSE*: This comment refers to a sentence in Section 2.4 (Respiratory): "Based on a systematic evaluation of the literature, respiratory toxicity is a suspected target of exposure to 4-nitrophenol." The word "target" was replaced with "health effects", and the sentence now reads: "Based on a systematic evaluation of the literature, respiratory toxicity is a suspected health effect of exposure to 4-nitrophenol."*

COMMENT: In average?

RESPONSE*: This comment refers to a sentence in Section 2.4 (Respiratory): "Increases in respiration rates of: 31% for 2-nitrophenol, 24% for 3-nitrophenol, and 2% for 4-nitrophenol (compared with a prior control period) were reported (Grant 1959)." The word "Average" was added to this sentence to clarify the described increases are average increases. The sentence now reads: "Average increases in*

respiration rates of: 31% for 2-nitrophenol, 24% for 3-nitrophenol, and 2% for 4-nitrophenol (compared with a prior control period) were reported (Grant 1959)."

COMMENT: Consider there is no change?

***RESPONSE**: This comment refers to a sentence in Section 2.4 (Respiratory): "Increases in respiration rates of: 31% for 2-nitrophenol, 24% for 3-nitrophenol, and 2% for 4-nitrophenol (compared with a prior control period) were reported (Grant 1959). However the toxicological significance of these findings is unclear." In response to this comment, we added an additional sentence that explains a control group also experienced a 2% increase in respiration rate, suggesting the change observed in rats injected with 4-nitrophenol is not significant. The updated sentences now read: "Average increases in respiration rates of: 31% for 2-nitrophenol, 24% for 3-nitrophenol, and 2% for 4-nitrophenol (compared with a prior control period) were reported (Grant 1959). However, a control group that received an intraperitoneal injection of normal saline also experienced a 2% increase in respiration rate compared to the prior control period, which suggests the small increase in respiration rate observed in rats injected with 4-nitrophenol is not significant (Grant 1959). The toxicological significance of these findings is unclear."*

COMMENT: I only see one study (Tang et al. 2016)

***RESPONSE**: This comment refers to a sentence in Section 2.6 (Gastrointestinal): "However, these studies were the only two acute oral studies to investigate gastrointestinal effects." There are two studies contained within the Tang et al. (2016) publication. As such, language was added to clarify. The sentence now reads: "However, these two studies presented in Tang et al. (2016) were the only acute oral studies to investigate gastrointestinal effects."*

COMMENT: Please provide the population size to interpret a possible lack of power.

***RESPONSE**: This comment refers to the following sentence in Section 2.7 (Hematological – Inhalation): "However, no statistically significant increases in methemoglobin levels occurred in the higher dose groups, which brings into question the validity of the finding in the 5 mg/m3 group (Hazleton 1984)." Population size information is contained within the Hazleton (1984) record in Table 2-2. Thus, no changes were made to the profile based on this comment.*

COMMENT: To interpret this results in terms of clinical impact, it would be necessary to provide the methemoglobin level before and after (a 200% increased of a very low level would have limited impact)

***RESPONSE**: This comment refers to a group of sentences in Section 2.7 (Hematological): "After exposing albino rats to 4-nitrophenol concentrations of 0, 26, or 112 mg/m^3 for 6 hours/day for 10 days, Smith et al. (1988) found that methemoglobin increased by 200% in males in the 112 mg/m^3 concentration group. However, the levels returned to normal after a 14-day recovery period; the NOAEL for these effects was 26 mg/m^3." In response to this comment, we added the specific methemoglobin levels that were presented in the study to clarify the magnitude of the 200% increase. As such, the revised sentences read: "After exposing albino rats to 4-nitrophenol concentrations of 0, 26, or 112 mg/m^3 for 6 hours/day for 10 days, Smith et al. (1988) found that methemoglobin increased by 200% in males in the*

112 mg/m^3 concentration group (1.5% methemoglobin in the 112 mg/m^3 group versus 0.5% methemoglobin in the control group). However, the levels returned to normal after a 14-day recovery period (0.2% methemoglobin); the NOAEL for these effects was 26 mg/m^3."

COMMENT: See supra: please provide the level of methemoglobin.

***RESPONSE**: This comment refers to a group of sentences in Section 2.7 (Hematological): "Another study within this same publication that exposed albino rats to 4-nitrophenol concentrations of 0, 294, and 2,133 mg/m^3 for 10 days also found that methemoglobin levels increased by 665%; the NOAEL for these effects was 294 mg/m^3. After 14 days of recovery, methemoglobin levels remained elevated by 250%." Revisions were made to these sentences to specify the levels of methemoglobin to clarify the 665% increase. As such, the revised sentence reads: "Another study within this same publication that exposed albino rats to 4-nitrophenol concentrations of 0, 294, and 2,133 mg/m^3 for 10 days also found that methemoglobin levels increased by 665% (1.53% methemoglobin in the 2,133 mg/m^3 group versus 0.20% methemoglobin in the control group); the NOAEL for these effects was 294 mg/m^3. After 14 days of recovery, methemoglobin levels remained elevated by 250% (0.70% methemoglobin)."*

COMMENT: Unclear to me. Is it effect on newborn that was prenatally exposed, or effect on young rats that were exposed just after birth?

***RESPONSE**: This comment refers to a sentence in Section 2.10 (Renal): Koizumi et al. (2001) also observed no renal effects of daily oral gavage doses of 4-nitrophenol for 18 days at doses up to 160 mg/kg/day in newborn Sprague-Dawley rats." This sentence was clarified in response to this comment by specifying that the rats were exposed after birth. The revised sentence reads: "Koizumi et al. (2001) also observed no renal effects of daily oral gavage doses of 4-nitrophenol for 18 days (days 4 through 21 after birth) at doses up to 160 mg/kg/day in newborn Sprague-Dawley rats."*

COMMENT: I agree this is a clear limitation. However, such effect appear unusual might be related to dermal applications.

***RESPONSE**: This comment refers to a sentence in Section 2.11 (Dermal): "However, the results of this study should be interpreted with caution as the study did not include a control group, which calls into question the rigor with which the study was conducted." Despite agreement with the reviewer's comment that the effect might be related to dermal application, it is impossible to know for sure without comparison to an unexposed group of rabbits. Thus, no changes were made to the profile based on this comment.*

COMMENT: This assumption appear subjective and inappropriate.

If the study did not match the minimum quality criteria, this should be excluded. Otherwise, please suppress this assumption.

***RESPONSE**: This comment refers to a sentence in Section 2.11 (Dermal): "However, the results of this study should be interpreted with caution as the study did not include a control group, which calls into question the rigor with which the study was conducted." This sentence has been revised based on the*

comment, and now reads: "However, the results of this study should be interpreted with caution as the study did not include a control group."

COMMENT: In the REPRODUCTIVE section, there is two study (Li et al. (2006) & Li et al. (2009)) suggesting a possible estrogenic effect of PNP.

These data should be move here, and the probability of an endocrine disrupting property of 4-nitrophenol should be debated.

***RESPONSE**: This comment refers to Section 2.13 (Endocrine). In response to this comment, all endocrine effects that were presented in Section 2.16 (Reproductive) were also added to the end of Section 2.13 (Endocrine). As was suggested in Section 2.16, given the lack of corroborating evidence of the observed endocrine outcomes in the presented studies using routes of exposure sufficient for MRL derivation, the results presented should be treated with caution regarding making generalizations about the effects of 4-nitrophenol on endocrine toxicity.*

The added language reads as follows: "Although there is no evidence of endocrine toxicity after exposure to 4-nitrophenol through oral, inhalation, or dermal routes, 4-nitrophenol has been shown to alter endocrine function after parenteral exposure in both male and female rodents. Li et al. (2006) showed that male rats exposed to 0.1 mg/kg/day 4-nitrophenol for 7 days via subcutaneous injections exhibited a significant increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the plasma at 0.1 mg/kg/day; this indicates that 4-nitrophenol has estrogenic and anti-androgenic activities in vivo. Li et al. (2009) also showed that acute exposure to 4-nitrophenol by subcutaneous injections at 0.01 mg/kg/day altered the plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone in male rats (X. Li et al., 2009). Zhang et al. (2017) showed that a single exposure to 4-nitrophenol via subcutaneous injections to neonatal female rats (treated at post-natal day [PND] 0) at 10 mg/kg/day potentially affects the expression of estrogen receptor β (ERβ) in the rat ovaries, resulting in the disrupted steroidogenesis during ovarian development and the delayed puberty. Zhang et al. (2013) also demonstrated that a daily exposure by subcutaneous injections for 4 weeks in male rats to a dose of 1 mg/kg 4-nitrophenol increased serum testosterone and hyperplasia of Leydig cells in the testes.

Zhang et al. (2013) observed a significant decrease in the levels of estradiol and aromatase expression along with an increase in the expression of the estrogen receptors α and β after a daily 4-week exposure to 10 mg/kg/day 4-nitrophenol. Zhang et al. (2015) observed that intermediate exposure to 100 mg/kg/day 4-nitrophenol by subcutaneous injection resulted in a significant decrease in sperm counts and serum testosterone levels, as well as morphological changes in the testes (Y. Zhang et al., 2015).

Given the lack of corroborating evidence of these observed endocrine outcomes in studies using the oral, inhalation, and dermal routes of exposure, these results should be treated with caution regarding making generalizations about the effects of 4-nitrophenol on endocrine toxicity."

COMMENT: Endocrine effects might be very variable between studies. Please defined which endocrine effect was screened in the different studies (which hormone…etc.), to identify possible gap in knowledge

***RESPONSE**: This comment refers to a sentence in Section 2.13 (Endocrine): "Hazleton (1983) found no endocrine effects of intermittent inhalation exposure to 4-nitrophenol for 4 weeks in rats at*

concentrations up to 30 mg/m^3." The specific endocrine effects that were monitored in each study in Section 2.13 have been added to provide additional information to the reader based on this comment.

COMMENT: Please provide the size of the group (lack of power?).

***RESPONSE**: This comment refers to a sentence in Section 2.16 (Reproductive): "There were no reproductive effects observed in female mice after acute gavage administration of 4-nitrophenol at 400 mg/kg/day for 8 days, though there was a slight non-statistically significant reduction in the average number of live pups per litter (Plasterer et al., 1985)." The size of each dose group is listed in the Plasterer et al. (1985) record in Table 2-2. Thus, no changes were made to the profile based on this comment.*

COMMENT: To be move to ENDOCRINE section?

***RESPONSE**: This comment refers to a sentence in Section 2.16 (Reproductive – Other): "Zhang et al. (2017) also provides evidence that acute exposure to 4-nitrophenol potentially affects the expression of estrogen receptor β (ERβ) in the rat ovaries, resulting in the disrupted steroidogenesis during ovarian development and the delayed puberty (Zhang et al. 2017)." This study could be present in either the endocrine section, or the reproductive section. The Zhang et al. (2017) study authors specify that the study was initiated to investigate the potential mechanism underlying the effects of neonatal exposure to 4-nitrophenol on the reproductive system in developing rats. As such, we have opted to keep this study in the reproductive section per ATSDR profile guidance, as the study seems to focus on whether or not there are effects on the reproductive system. However, we have also added a mention of this study to the endocrine section.*

COMMENT: To be move to ENDOCRINE section?

***RESPONSE**: This comment refers to a sentence in Section 2.16 (Reproductive – Other): "Zhang et al. (2013) observed a significant decrease in the levels of estradiol and aromatase expression along with an increase in the expression of the estrogen receptors α and β after a daily 4-week exposure to 10 mg/kg/day 4-nitrophenol (Zhang et al. 2013)." This study could be present in either the endocrine section, or the reproductive section. The Zhang et al. (2013) study authors specify that the study was initiated to investigate 4-nitrophenol's reproductive effects in immature male rats. As such, we have opted to keep this study in the reproductive section per ATSDR profile guidance, as the study seems to focus on whether or not there are effects on the reproductive system. However, we have also added a mention of this study to the endocrine section.*

COMMENT: I am feeling a bit uncomfortable to simply put apart five recent publications because these were not matching previous results.

At least justified or discussed why studies based on subcutaneous injections are systematically inconsistent (different mechanisms suspected, difference in bioavailability, difference in protocol / species…).

RESPONSE*: This comment refers to a sentence in Section 2.16 (Reproductive – Other): "Given the lack of corroborating evidence of these observed reproductive outcomes in studies using the oral, inhalation, and dermal routes of exposure, these results should be treated with caution regarding making generalizations about the effects of 4-nitrophenol on reproductive toxicity." These studies are not set aside because they do not match previous results, as the reviewer suggested. This sentence was included in the profile because these studies used a route of exposure that is generally considered by ATSDR only to be supporting evidence to studies using the inhalation, oral, or dermal routes. Thus, although these studies provide some evidence that 4-nitrophenol could cause reproductive effects after intraperitoneal injections, it is unclear whether these effects would persist if exposure were to occur via the inhalation, oral, or dermal route. As such, no changes were made to the profile based on this comment.*

COMMENT: Is it possible to provide here a range of half-lives values obtained?

i.e: observed half-lives for 4-nitrophenol ranged from 0.6 to 5h depending on species, exposure pathways and sex in the animal literature.

RESPONSE*: The comment refers to the introductory summary regarding "Excretion" at the beginning of Section 3.1 (Toxicokinetics). In response to this comment, a sentence was added providing the range of observed half-life values in the animal literature. The added sentence reads: "Observed half-life values for 4-nitrophenol ranged from 0.6 to 5 hours depending on species, exposure pathways, and sex in the animal literature."*

COMMENT: This is a very light hypothesis…

Systemic effect or metabolites might impact these organs without specific distribution of PNP.

RESPONSE*: This comment refers to a sentence in Section 3.1.2 (Distribution): "However, in one study, Smith et al. (1988) reported a decrease in lung and spleen weight after inhalation exposure to 4-nitrophenol for 14 days, which could indicate the potential distribution of 4-nitrophenol to these organs." This sentence was deleted in response to this comment.*

COMMENT: How to be sure that it was related to excretion, and not a simple bioaccumulation due to the physicochemical properties of PNP?

RESPONSE*: This comment refers to a sentence in Section 3.1.2 (Distribution): "These results suggest that while the majority of 4-nitrophenol was distributed to the gastrointestinal tract for excretion, the liver and kidney may also play roles in the metabolism of 4-nitrophenol after oral exposure." In response to this comment, the words "for excretion" were removed from this sentence, and the resulting sentence reads: "These results suggest that while the majority of 4-nitrophenol was distributed to the gastrointestinal tract, the liver and kidney may also play roles in the metabolism of 4-nitrophenol after oral exposure."*

COMMENT: I guess some difference might be expected between animal species regarding metabolism pathways. Maybe few word about such uncertainty might be added to this part (+/- some pathway expected to be under or over expressed in human, compared to rabbits / rodents).

RESPONSE*: This comment refers to Section 3.1.3 (Metabolism). In response to this comment, a sentence was added to the end of the introductory paragraph that reads: "As metabolic information on nitrophenols comes exclusively from animal studies, some differences may be anticipated regarding how this information generalizes to metabolism pathways in humans."*

COMMENT: Please indicate which species.

RESPONSE*: This comment refers to a sentence in Section 3.1.3 (Metabolism): "4- Nitrophenol is rapidly metabolized in animals after dermal exposure." The specific animals were added, and the sentence now reads: "4- Nitrophenol is rapidly metabolized in dogs, rabbits, and pigs after dermal exposure."*

COMMENT: Some other chemicals such as glycol ethers are using the same metabolism pathways than ethanol.

Do you think that exposure to such chemicals might favoring the formation of 4-nitrocatechol?

RESPONSE*: This comment refers to a sentence in Section 3.2 (Children and Other Populations that are Unusually Susceptible): "Based on results from a study of ethanol-treated rats, it is possible that individuals who consume ethanol may have slower rates of clearance of 4-nitrophenol due to the presence of ethanol causing rapid metabolization of 4-nitrophenol into 4-nitrocatechol, which competes with 4-nitrophenol for the formation of sulfate and glucuronide conjugates (Reinke & Moyer, 1985)." It is possible it is the case that exposure to chemicals such as glycol ethers might favor the formation of 4-nitrocatechol, however this type of information is beyond the scope of information to be included for this profile, as it is specific to other chemicals aside from nitrophenols. Thus, no changes were made to the profile based on this comment.*

COMMENT: The fetus as well… If nitrophenols are crossing the placental barrier, I guess that the fetus might be impacted.

Concerning the newborn, in my knowledge, fetal hemoglobin was rapidly eliminated and replaced after birth.

RESPONSE*: This comment refers to a sentence in Section 3.2 (Other Children and Other Populations that are Unusually Susceptible): "Newborn infants utilize fetal hemoglobin, which has reduced oxygen-carrying capacity, and also have low levels of NADPH, which continuously reduces methemoglobin. Therefore, infants (as well as individuals congenitally deficient in this enzyme) may represent unusually susceptible subpopulations (Naoum, 2012)." This language was updated to specify that newborn infants utilize fetal hemoglobin until they are 2-4 months old. A mention of pregnant women and their fetuses being a potentially susceptible subpopulation was also added. The updated sentences now read: "Newborn infants utilize fetal hemoglobin until they are 2-4 months old and have reduced oxygen-carrying capacity (Schechter, 2008). Infants also have low levels of NADPH, which continuously reduces methemoglobin. Therefore, infants (as well as individuals congenitally deficient in this enzyme), and potentially pregnant women and their fetuses, may represent unusually susceptible subpopulations, as there is some evidence to suggest that 4-nitrophenol crosses the placental barrier (Abu-Qare et al. 2000; (Naoum, 2012))."*

COMMENT: Suppress from the list (redundant)?

RESPONSE*: This comment refers to a sentence in Section 3.2 (Children and Other Populations that are Unusually Susceptible): "Exposure to xenobiotics like aniline, chlorobenzene, fires, organic nitrites, nitrophenols, and nitrites and nitrates from well water and food, respectively, are all implicated in causing acquired methemoglobinemia." In response to this comment, the word "nitrophenols" has been removed from this list. The updated sentence reads: "Exposure to xenobiotics like aniline, chlorobenzene, fires, organic nitrites, and nitrites and nitrates from well water and food, respectively, are all implicated in causing acquired methemoglobinemia."*

COMMENT: It is the same for the hair matrix.

RESPONSE*: This comment refers to a sentence in section 3.3.1 (Biomarkers of Exposure): "While the presence of 4-nitrophenol in the urine may be due to exposure to 4-nitrophenol itself, it may also be the result of exposure to other chemicals such as methyl parathion, and nitrobenzene, of which 4-nitrophenol is a metabolite ((Barr et al., 2002); (EPA, 2009); (A. J. Li & Kannan, 2018; Y. Li et al., 2019)), confounding its use as a biomarker of exposure." Based on this comment, a sentence was added at the end of this section introducing hair as a potential biomarker that suffers from similar confounding issues as urine. This added sentence reads:" Hair has been used as a biomarker of exposure to 4-nitrophenol that captures cumulative exposure over a longer period of time, but these levels could also be due to exposure to 4-nitrophenol or those for which 4-nitrophenol is a metabolite, and more research is needed to understand the correlation of hair measurements with serum or urine concentrations of 4-nitrophenol (Beranger et al. 2018)."*

COMMENT: This is not the case for hair.

Even if further research are needed to assess precisely the stability over time and the correlation with serum / urine concentration, hair is suspected to archive exposure for a longer time period. In the study from Béranger et al. (2018), the authors measured cumulative exposures from the 9 previous months.

RESPONSE*: : This comment refers to a sentence in section 3.3.1 (Biomarkers of Exposure): "Due to the rapid excretion of 2- and 4-nitrophenol conjugates in the urine, their use as biomarkers of exposure may be limited to acute exposures only." Based on this comment, a sentence was added at the end of this section introducing hair as a potential biomarker that suffers from similar confounding issues as urine. This added sentence reads:" Hair has been used as a biomarker of exposure to 4-nitrophenol that captures cumulative exposure over a longer period of time, but these levels could also be due to exposure to 4-nitrophenol or those for which 4-nitrophenol is a metabolite, and more research is needed to understand the correlation of hair measurements with serum or urine concentrations of 4-nitrophenol (Beranger et al. 2018)."*

COMMENT: As presented, this figure would suggest differences in contamination between states.

However, since the true number of hazardous waste site a not known, it does not correspond to the true distribution of contaminated site. Also, all the state are not covering the same surface.

I assume it would be more informative to provide the proportion of NPL contaminated site in each area, or the number of contaminated site per standardize surface unit.

***RESPONSE**: This comment refers to Figure 5-1 in Section 5.1 (Overview). This figure is a standard figure across all ATSDR Toxicological Profiles and is defined as such based on the ATSDR Profile Development Guidance. As such, no changes were made to the profile based on this comment.*

COMMENT: It would be informative to provide the maximum level (or the 95e percentile), to interpret the maximum level that can be expected in the environment.

***RESPONSE**: This comment refers to a sentence in Section 5.3.1 (Air): "2-Nitrophenol and 4-nitrophenol were not detected in the emissions from the burning of three types of firewood, but 4-nitrophenol was detected at an average concentration of 0.09±0.08 µg/m^3 in emissions from pellet stoves (Rubio et al., 2019)." As the referenced study does not include a maximum or 95th percentile value for these emissions, no changes were made to the profile based on this comment.*

COMMENT: Which ones?

***RESPONSE**: This comment refers to a sentence in Section 5.4.1 (Transport and Partitioning): "The log Koc values of 2.06-2.42 can be used to predict that the two nitrophenols would not be sorbed appreciably to sediments." The words "the two nitrophenols" were referring to 2- and 4-nitrophenol. Thus, based on this comment, the sentence was updated to read: "The log Koc values of 2.06-2.42 can be used to predict that 2- and 4-nitrophenol would not be sorbed appreciably to sediments."*

COMMENT: 2- and 4-nitrophenol?

***RESPONSE**: This comment refers to a sentence in section 5.4.2 (Water): "Chemical oxidation reactions of the two nitrophenols by singlet oxygen and alkyl peroxy radicals formed as a result of sunlight-induced photochemical reactions in water are too slow to be significant." 2- and 4-nitrophenol were added, and the sentence now reads: "Chemical oxidation reactions of 2- and 4-nitrophenol by singlet oxygen and alkyl peroxy radicals formed as a result of sunlight-induced photochemical reactions in water are too slow to be significant."*

COMMENT: 10^6?

***RESPONSE**: This comment refers to a sentence in section 5.4.2 (Water): "… and a mean of less than one day for five pond and river waters based on the concentration of degrader microorganisms of 1O+6 organisms/ml." The format was fixed and the sentence now reads: "… and a mean of less than one day for five pond and river waters based on the concentration of degrader microorganisms of 10^6 organisms/ml."*

COMMENT: Table 5.3?

***RESPONSE**: This comment refers to a sentence in section 5.5 (Levels in the Environment): "Table 5-5 shows the limit of detections typically achieved by analytical analysis in environmental media." Table 5-5 was replaced by the correct table, Table 5-3, and the sentence now reads: "Table 5-3 shows the limit of detections typically achieved by analytical analysis in environmental media."*

COMMENT: Table 5.4?

***RESPONSE**: This comment refers to a sentence in section 5.5 (Levels in the Environment): "Presented in Table 5-6 is a summary of the range of concentrations detected in environmental media at NPL sites." Table 5-6 was replaced by the correct table, Table 5-4, and the sentence now reads: "Presented in Table 5-4 is a summary of the range of concentrations detected in environmental media at NPL sites."*

COMMENT: It would be informative to provide the maximum level (or the 95e percentile), to interpret the maximum level that can be expected in the environment.

***RESPONSE**: This comment refers to Table 5-4 in Section 5.5 (Levels in the Environment). Although including the maximum level or 95th percentile may be informative to include, this table is a standard table across all ATSDR Toxicological Profiles and is defined as such based on the ATSDR Profile Development Guidance. As such, no changes were made to this table based on this comment.*

COMMENT: The number is not following 5.4 => 5.7

***RESPONSE**: Table numbering was adjusted.*

COMMENT: Please indicate in when the parathion was banned in the US, this would help to interpret data on environmental and biological monitoring (depending if the sampling was conducted before or after the date of banishment).

***RESPONSE**: This comment refers to a sentence in Section 5.5.4 (Other Media): "The production of 4-nitrophenol from degradation or metabolism of several pesticides, including parathion (which is no longer used in the U.S.) and methyl parathion, on plant leaves or in soil may result in the contamination of food crops following application of these pesticides." In response to this comment, the date of last legal use of parathion was added to this sentence. As such, the revised sentence now reads: "The production of 4-nitrophenol from degradation or metabolism of several pesticides, including parathion (which is no longer used in the U.S. as of October 2003) and methyl parathion, on plant leaves or in soil may result in the contamination of food crops following application of these pesticides."*

COMMENT: Please check the table numbering.

***RESPONSE**: Table numbering was adjusted.*

COMMENT: The number is not following 5.8 => 5.5

***RESPONSE**: Table numbering was adjusted.*

COMMENT: Is it possible to add information on the detection frequency?

***RESPONSE**: This comment refers to Table 5-7 in Section 5.6 (General Population Exposure). Although adding information on the detection frequency may be informative to include, this table is a standard table across all ATSDR Toxicological Profiles and is defined as such based on the ATSDR Profile Development Guidance. No changes were made to this table based on this comment.*

COMMENT: And dermal?

***RESPONSE**: This comment is found in section 6.2 (Acute-Duration MRLs). MRLs are not developed for dermal exposures. Therefore, dermal was not mentioned in this sentence. No changes were made to the profile based on this comment.*

COMMENT: And dermal?

***RESPONSE**: This comment is found in section 6.2 (Intermediate-Duration MRLs). MRLs are not developed for dermal exposures. Therefore, dermal was not mentioned in this sentence. No changes were made to the profile based on this comment.*

COMMENT: In my opinion, additional data are also lacking regarding "endocrine" and "reproductive" effects. Studies based on subcutaneous injections have suggested estrogenic effects, as well as possible reproductive effect that potentially differs from other studies.

***RESPONSE**: The comment is found in Section 6.2 (Health Effects) regarding the following sentences: "There were a large number of intraperitoneal reproductive studies that showed potential effects in both male and female rats and mice, however, there were no studies using routes of exposure considered relevant for MRL derivation, such as inhalation, oral, or dermal exposure. A data need exists for the study of reproductive effects using these human-relevant routes of exposure." In response to this comment, the potential endocrine effects that were investigated in the previously mentioned intraperitoneal reproductive studies were mentioned as a data need in this section. Thus, these sentences were updated to read as follows: "There were a large number of intraperitoneal studies that showed potential reproductive/endocrine effects in both male and female rats and mice, however, there were no studies using routes of exposure considered sufficient for MRL derivation, such as inhalation, oral, or dermal exposure. A data need exists for the study of reproductive/endocrine effects using these human-relevant routes of exposure at exposure levels relevant for human populations."*

COMMENT: Some additional mechanism of action might be found using toxicogenomic approach.

Using the Comparative Toxicogenomic Database, it is possible identify genes associations to specific compounds and disease associated to these genes.

As an example, some direct evidence suggesting action on the liver injury are reported for 4-nitrophenol (http://ctdbase.org/detail.go?type=chem&acc=C024836&view=disease).

***RESPONSE**: This comment refers to Section 6.2 (Identification of Data Needs – Mechanisms of Action). The gene interactions that are discussed in the Comparative Toxicogenomic Database use either in vitro or animal models that are not considered relevant for developing the ToxProfiles as per the profile development guidance provided by ATSDR. Other metabolic information that is reported in this database, such as the involvement of CYP2E1 in metabolism is already included in the profile. Thus, no changes were made to the profile based on this comment.*

COMMENT: Data are also lacking regarding the possible distribution through the placental barrier, especially since fetal hemoglobin might be more sensitive to PNP.

***RESPONSE**: This comment refers to Section 6.2 (Identification of Data Needs – Absorption, Distribution, Metabolism, and Excretion). In response to this comment, a sentence was added to this section that reads: "A specific data need exists for further information regarding the possible distribution of 4-nitrophenol through the placental barrier, as fetal hemoglobin might be more sensitive to the effects of 4-nitrophenol."*

COMMENT: There enough data to assess adverse health effects for oral acute exposure to 4-nitrophenol. I assume it would be better to set up a MRL based on 200mg/kg/day, than not deriving MRL, even in absence of NOAEL.

***RESPONSE**: This comment refers to the Minimal Risk Level (MRL) Worksheet in Appendix A. It is ATSDR policy to not derive an MRL at a dose level where a serious effect has been observed. Since there are no other adequately conducted studies with observed health effects at acute oral doses of 4-nitrophenol below 200 mg/kg/day, we are unable to derive an acute oral MRL. No changes were made to the profile based on this comment.*

COMMENT: If the literature search was restricted to study published >1990, why several studies cited in the text were published in 70's, or in 80's?

***RESPONSE**: This comment refers to a sentence in Section B.1.1 (Literature Search): "The current literature search was intended to update the existing toxicological profile for nitrophenols (ATSDR 1992), thus, the literature search was restricted to studies published between January 1990 to June 2020." The updated literature search was restricted to studies published after the development of the previous version of the profile. However, studies included in the previous version of the profile were included in this version where appropriate. No changes were made to the profile based on this comment.*

COMMENT: Please specify if the manual screening was performed by a single or multiple investigator. If multiple investigators screened the same articles, how conflict in the selection process were solved? Is there any specific exclusion criteria used in the selection process (e.g. language, available abstract…)

RESPONSE*: This comment refers to Section B.1.2 (Literature Screening – Title and Abstract Screen). In response to this comment, a clarification was added that titles and abstracts were screened manually by a single reviewer for relevance. There were no additional exclusion criteria used in the selection process apart from being included based on the inclusion criteria listed in Table B-1.*

COMMENT: I don't understand why indicating this information…

RESPONSE*: This comment refers to the following bullet in Section B.1.2 (Literature Screening – Full Text Screen): "Number of studies cited in the pre-public draft of the toxicological profile: 45." The second bullet (quoted in the previous sentence) shows the number of studies cited in the draft toxicological profile, before it is posted for public comment. After the public comment and an additional literature review, more studies may be cited in the profile, so there is a third bullet to show the number of studies cited in the final profile.*

COMMENT: Some studies appeared on multiple line, with different rating. It is currently not possible to interpret to which results each rating refers.

RESPONSE*: This comment refers to Table C-4 in Appendix C. These are instances where multiple studies were included from a single reference. In these cases, we have further specified which sub-study the information refers to.*

Comments provided by Peer Reviewer #3

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: Not applicable.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: I think yes. Since some adverse health effects on aminals were observed and some animals were died in some studies, I think that we cannot exclude the adverse health effects to humans.

RESPONSE: *No revisions were suggested.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT: I agree that the data are insufficient to derive MRLs for all exposure routes and durations. In particular, there is no study to investigate any effect of nitrophenols on humans.

RESPONSE: *No revisions were suggested.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT: Not applicable.

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: Not applicable.

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: I think that MRL is properly assessed.

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Not applicable.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: I think yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT: Although only the animal data have been reported, I think that the dose-response relationships were well summarized in Tables 2-1, 2-2, and 2-3.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: I think that the NOAELs and/or LOAELs in the tables and the figures are appropriate. But some figures in the text seemed to be wrong. So, I revised or pointed out them. Please check them.

RESPONSE: *All instances where figures in the text contained inconsistencies were addressed. This includes Figure 2-X in Appendix D, where the inconsistent numbering was fixed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: I think yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: I think yes.

RESPONSE: *No revisions were suggested.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT: I think yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: I agree with the summary described in Section 3.1.6.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: Yes and I think that the discussions are well surmalized because the methemoglobinemia was suggested to link with the observed result in the animal study (the increase of methermoglobin).

RESPONSE: *No revisions were suggested.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT: I agree that 2- and 4-nitrophenol conjugates in the urine may be biomarkers of exposure for acute exposures due to the rapid excretion of them. But I think that further studies are needed to identify biomarkers of exposure for nitrophenols.

RESPONSE: *The point that further studies are needed to identify biomarkers of exposure for nitrophenols is discussed in Section 6.2 (Biomarkers of Exposure and Effect). In response to this comment, an additional sentence was added to Section 6.2 that reads: "A data need has been identified to determine biomarkers of exposure that are specific to nitrophenols."*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT: I agree with the discussion in Sec. 3.3.2. I think that further studies are needed to identify biomarkers of effect for nitrophenols, too.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: Yes, there is the adequate discussion. In the discussion, the studies regarding the attenuation of the effects of exposure to nitrophenols were summarized. I think that the sttenuation is expected at hazardous waste sites.

RESPONSE: *No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: Yes. It is written that 4-nitrophenol is rapidly converted to 4-nitrocatechol by ethanol and that arginine, quercetin, and phytosterol have antioxidant properties.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: I did not find any of wrong or missing values.

RESPONSE: *No revisions were suggested.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT: The chemical and physical properties of three isomers of nitrophenols are provided.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: I think yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes.

I think yes.

I have no additional information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes.

I have no additional information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes. But I found some mistakes in the text. I pointed out, so please check them.

Yes.

Yes.

Yes.

I have no additional information.

RESPONSE: *We have checked the numbers presented in Section 5.5.2 (Water) related to information extracted from STORET, and have verified that the original numbers presented in the profile are accurate. As such, no changes were made to the profile based on this comment.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: Yes.

Yes.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT: I have no idea.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT: Yes. I think that the Sec. 6.2. is well summarized.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT: I did not feel any bias in the text.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT: I have no idea.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

Additional References from Reviewer*

**These are references cited within the reviewer's individual comments. Responses to the reviewer's comments specify the disposition of these references within the toxicological profile.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT: Yes.

RESPONSE: *No revisions were suggested. Responses to further comments follow.*

Unpublished Studies (If Applicable to Review)

COMMENT: Reviewer 1 comments on "Monsanto. 1990. Range-Finding Teratology Study In Rats. St. Louis, MO." can be found in the attached file named "Reviewer 1 Comments Unpublished Study.doc".

RESPONSE: *Based on the reviewer comments, the aforementioned study has been included in the profile.*

Annotated Comments on the Profile

COMMENT: Bingham et al. 2006 is not listed in References.

RESPONSE*: Bingham et al. 2006 was added in the References section and the formatted citation was added into the text.*

COMMENT: The explanations of DX, FI, and RX are missing.

RESPONSE*: Included the explanations for DX = developmental toxicity, FI = Food Intake, and RX= reproductive toxicity.*

COMMENT: The explanations of DX, OW, and RX are missing.

RESPONSE*: This comment is found in table 2-3 on page 34. Change was made as requested and the following explanations were included: DX = developmental toxicity, OW = organ weight, and RX= reproductive function.*

COMMENT: Is it 1000 according to Table 2-2?

RESPONSE*: This comment refers to a sentence in Section 2.3 (Body Weight): "Intermediate duration exposure to 4-nitrophenol showed no body weight effects following daily doses by gavage of up to 400 mg/kg/day in Sprague-Dawley rats for 18 days, 28 days, or 13 weeks ((Hazleton, 1989); (Koizumi et al., 2001)). The 400 mg/kg/day was a typo, and was replaced with 1,000 mg/kg/day. With this change, the sentence now reads: "Intermediate duration exposure to 4-nitrophenol showed no body weight effects following daily doses by gavage of up to 1,000 mg/kg/day in Sprague-Dawley rats for 18 days, 28 days, or 13 weeks ((Hazleton, 1989); (Koizumi et al., 2001)).")*

COMMENT: Is it 1000 according to Table 2-2?

RESPONSE*: This comment refers to a sentence in Section 2.6 (Gastrointestinal): "Intermediate oral exposure to 4-nitrophenol by gavage at doses up to 400 mg/kg/day for 28 days or at doses up to 140 mg/kg/day for 13 weeks showed no significant effects, though it is unclear that these studies looked specifically at the gastrointestinal endpoints observed in Tang et al. (2016) ((Hazleton, 1989); (Koizumi et al., 2001))." The 400 mg/kg/day in this sentence was a typo, and was replaced with 1,000 mg/kg/day. Thus, the revised sentence now reads: "Intermediate oral exposure to 4-nitrophenol by gavage at doses up to 1,000 mg/kg/day for 28 days or at doses up to 140 mg/kg/day for 13 weeks showed no significant effects, though it is unclear that these studies looked specifically at the gastrointestinal endpoints observed in Tang et al. (2016) ((Hazleton, 1989); (Koizumi et al., 2001)).")*

COMMENT: Robinson et al. 1951a or 1951b?

RESPONSE*: This comment refers to a sentence in Section 3.1.1 (Absorption): "Other oral dosed studies that measured metabolites in the excreta indicate absorption of 4-nitrophenol (Robinson, Smith, & Williams, 1951; Williams, 1938). The correct citation was added into the sentence, which now reads:*

"Other oral dosed studies that measured metabolites in the excreta indicate absorption of 4-nitrophenol (Robinson et al., 1951; Williams, 1938)."

COMMENT: 1951a or 1951b?

***RESPONSE**: This comment refers to a sentence in Section 3.1.3 (Metabolism): "In these rabbits, conjugation was almost complete with 70% of the dose excreted in urine being in the form of nitrophenol glucuronides (Robinson et al. 1951). The correct citation was added into the sentence, which now reads: "In these rabbits, conjugation was almost complete with 70% of the dose excreted in urine being in the form of nitrophenol glucuronides (Robinson et al. 1951a).*

COMMENT: 1951a or 1951b?

***RESPONSE**: This comment refers to a sentence in Section 3.1.3 (Metabolism): "80% of the nitro group of the nitrophenols was excreted in urine and unchanged; the rest underwent reduction ranging from 6-14% of the dose (Robinson et al. 1951)." The correct citation was added into the sentence, which now reads: "80% of the nitro group of the nitrophenols was excreted in urine and unchanged; the rest underwent reduction ranging from 6-14% of the dose (Robinson et al. 1951a)."*

COMMENT: 1951a or 1951b?

***RESPONSE**: This comment refers to a sentence in Section 3.1.3 (Metabolism): "Oxidation accounted for less than 1% of the dose (Robinson et al. 1951)." The correct citation was added into the sentence, which now reads: "Oxidation accounted for less than 1% of the dose (Robinson et al. 1951a)."*

COMMENT: This paper is not listed in References.

***RESPONSE**: Krishnan et al. 1994 was added in the References section and the formatted citation was added into the text.*

COMMENT: Is this "Table 5-7"?

***RESPONSE**: This comment refers to a sentence in Section 3.3.1 (Biomarkers of Exposure): "Based on the current body of literature, it is not known if urinary excretion of 2- or 4-nitrophenol (or their conjugates) can be associated quantitatively with exposure to these chemicals. NHANES data (see Table 5-1) identifies the levels of 4-nitrophenol in urine; however, this could be due to exposure to 4-nitrophenol itself or to a chemical that is metabolized to 4-nitrophenol." Table 5-1 was replaced by the correct table, Table 5-7, and the sentence now reads: "Based on the current body of literature, it is not known if urinary excretion of 2- or 4-nitrophenol (or their conjugates) can be associated quantitatively with exposure to these chemicals. NHANES data (see Table 5-7) identifies the levels of 4-nitrophenol in urine; however, this could be due to exposure to 4-nitrophenol itself or to a chemical that is metabolized to 4-nitrophenol."*

COMMENT: Bingham et al. 2006 is not listed in References.

RESPONSE*: Bingham et al. 2006 was added in the References section and the formatted citation was added into the text.*

COMMENT: Is this correct?

RESPONSE*: No revisions needed.*

COMMENT: Lower than?

RESPONSE*: This comment refers to a sentence in Section 5.4.1 (Transport and Partitioning): "The dissociation constant (pKa) values of the two compounds (7.23 for 2-nitrophenol and 7.15 for 4-nitrophenol) indicate that significant fractions of these nitrophenols will be dissociated at pHs above 6." The sentence was confusing as written, and was revised and now reads: "The dissociation constant (pKa) values of the two compounds (7.23 for 2-nitrophenol and 7.15 for 4-nitrophenol) indicate that significant fractions of these nitrophenols will exist in partially anion form in the environment."*

COMMENT: This paper is not listed in References.

RESPONSE*: Geyer et al. 1984 was added in the References section and the formatted citation was added into the text.*

COMMENT: Am I correct?

RESPONSE*: This comment refers to a sentence in Section 5.5.2 (Water): "Concentrations in these samples ranged from 0.08-1,900 µg/L (4-nitrophenol) and 0.02-790 µg/L (2-nitrophenol)." The original numbers are correct, according to the data. It can be reproduced by filtering on surface water (column F, ActivityMediaSubdivisionName), o- or p-nitrophenol (column AF, CharacteristicName), and NWIS (column BK, ProviderName).*

COMMENT: Am I right?

RESPONSE*: This comment refers to a sentence in Section 5.5.2 (Water): "Concentrations of 2-nitrophenol and 4-nitrophenol were 0-10,000 µg/L and <0.6-25000 µg/L, respectively (WQP 2020)." The original numbers are correct, according to the data. It can be reproduced by filtering on ground water and groundwater (column F, ActivityMediaSubdivisionName), o- or p-nitrophenol (column AF, CharacteristicName), and NWIS (column BK, ProviderName). However, while checking this data, the data from the previous sentences was found to be incorrect and has been changed.*

COMMENT: When?

***RESPONSE**: This comment refers to Table 5-6 in Section 5.5.3 (Sediment and Soil). The correct year is 2003. This change has been made.*

COMMENT: Am I correct?

***RESPONSE**: This comment refers to a sentence in Section 5.5.3 (Sediment and Soil): "3-Nitrophenol was detected in 32 of 80 sediment samples collected between 1992 and 2011 for WQP at concentrations ranging from 17 to 3,800 µg/kg (WQP 2020)." The original numbers are correct, according to the data. It can be reproduced by filtering on sediment (column E, ActivityMediaName).*

COMMENT: It is better to start new page.

***RESPONSE**: Suggestion accepted.*

COMMENT: Some papers are not cites in the text. So, I deleted them. Please check if it is correct.

***RESPONSE**: We have updated the references section to reflect all studies cited in the profile.*

COMMENT: Branch and Stout 1983b?

***RESPONSE**: This comment refers to a sentence in Appendix A: "There are also other system respiratory effects that have been observed after oral exposure to 4-nitrophenol such as wheezing and dyspnea (Branch 1983a, Hazleton 1989), but these effects were observed for a different route of exposure." The correct reference, Branch and Stout 1983a, has been added to the sentence.*

COMMENT: New paper cited in Appendix A.

***RESPONSE**: This comment refers to a sentence in Appendix A: "Since death is always considered a serious effect, this precludes the derivation of an intermediate oral MRL at this dose (ATSDR 2018)." ATSDR 2018 was added in the References section and the formatted citation was added into the text.*

COMMENT: New paper cited in Appendix B.

***RESPONSE**: This comment refers to a sentence in Section B.1.1 (Literature Search): "The current literature search was intended to update the existing toxicological profile for nitrophenols (ATSDR 1992), thus, the literature search was restricted to studies published between January 1990 to June 2020." ATSDR 1992 was added in the References section and the formatted citation was added into the text.*

COMMENT: New papers cited in Appendix C.

***RESPONSE**: This comment refers to a sentence in Appendix C: "To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the*

health effects associated with exposure to nitrophenols, ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014)." NTP 2013, NTP 2015b, and Rooney et al. 2014 were added in the References section and the formatted citations were added into the text.

COMMENT: New papers cited in Appendix C.

***RESPONSE**: This comment refers to a sentence in Section C.2.1 (Literature Search): "The current literature search was intended to update the existing toxicological profile for nitrophenols (ATSDR 1992), thus, the literature search was restricted to studies published between January 1990 to June 2020." ATSDR 1992 was added in the References section and the formatted citation was added into the text.*

COMMENT: ?

***RESPONSE**: This comment refers to a sentence in Section C.2.1 (Literature Search): "See 0 Appendix B for the databases searched and the search strategy." The "0" was removed and the sentence now reads: "See Appendix B for the databases searched and the search strategy."*

COMMENT: New paper cited in Appendix C.

***RESPONSE**: This comment refers to a sentence in Section C.5.1 (Risk of Bias Assessment): "The risk of bias of individual studies was assessed using OHAT's Risk of Bias Tool (NTP 2015)." NTP 2015a was added in the References section and the formatted citation was added into the text.*

COMMENT: Header should be "APPENDIX C" and Page number should be "C-18". These mistakes continue up to page 23.

***RESPONSE**: The headers have been fixed.*

COMMENT: New paper cited in Appendix D.

***RESPONSE**: This comment refers to a sentence in Appendix D: "MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure." Barnes and Dourson 1988 was added in the References section and the formatted citation was added into the text.*

COMMENT: Header should be "APPENDIX D" and Page number should be "D-2". Such mistakes continue up to p199

***RESPONSE**: The headers have been fixed.*

COMMENT: New paper cited in Appendix D.

RESPONSE*: This comment refers to a sentence in Appendix D: "For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992)." This reference exists just as an example of how a reference paper will show up in the profile. No changes were made to the profile based on this comment.*

COMMENT: The numbers in square are wrong. 13→12, 14→13, …, 18→17.

RESPONSE*: This comment refers to Figure 2-X in Appendix D. Thank you for pointing this out. This appendix has been updated so that the number 12 is no longer skipped in the figure, and the numbers in the list have been checked to confirm that they correspond to the numbers in the figures.*

COMMENT: Header should be "APPENDIX E" and Page number should be "E-2".

RESPONSE*: The headers have been fixed.*

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