DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR NITROBENZENE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Agency for Toxic Substances and Disease Registry

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

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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

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: I agree with the effects known to occur in humans as reported in the text.

RESPONSE: *No response needed.*

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: Effects observed in animals are largely consistent with effects in humans, particularly hematologic and respiratory system effects. Although evidence for liver and kidney effects in humans is limited, there is enough to suggest that liver and kidney effects in animals may be relevant to humans.

RESPONSE: *No response needed.*

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

COMMENT: For the purposes of this chapter, the overview of exposure conditions presented is adequate.

RESPONSE: *No response needed.*

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: The health effect conclusions are consistent with the published literature. The chapter clearly identifies the effects most consistently observed between animal and human studies, and the effects occurring at the lowest exposures, which is important for risk assessment (MRL derivation).

RESPONSE: *No response needed.*

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: As noted in the chapter, nitrobenzene toxicity information from humans comes almost exclusively from case reports and largely represents acute poisonings. Although this information is valuable from a hazard identification standpoint, the limitations of case studies are well understood and adequately addressed in the chapter.

RESPONSE: *No response needed.*

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: Adequately designed animal studies exist in the literature and are described in the text. Critical information regarding these studies is presented in tabular form and discussed in text. From these studies, adequate information is available to derive MRLs for inhalation and oral nitrobenzene exposure (except acute and chronic oral MRL).

RESPONSE: *No response needed.*

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: Most of the animal studies were conducted using mice and rats, with a few studies using rabbits. These are standard animal models for toxicity testing and appropriate for the endpoints of the studies and for the types of toxicity produced by nitrobenzene. There is no deficiency in the animal study database regarding species tested.

RESPONSE: *No response needed.*

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

COMMENT: The human data available for nitrobenzene are derived from case studies. These data are useful for hazard identification but not dose-response assessment. The focus of the dose-response assessment in this profile is, appropriately, on the animal data.

RESPONSE: *No response needed.*

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: As best I can determine, all of the relevant studies were included in the profile.

RESPONSE: *No response needed.*

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: This question does not seem relevant to nitrobenzene.

RESPONSE: *No response needed.*

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 didn't find any incorrect NOAELs or LOAELS in the figures, tables, or text.

RESPONSE: *No response needed.*

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: As noted in the profile, these decisions are pretty subjective, guidance from the agency on distinguishing "less serious" from "serious" notwithstanding. Although some of the calls could be questioned, there are none that I consider to be sufficiently wrong to recommend changing.

RESPONSE: *No response needed.*

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: There is generally little discussion of possible mechanisms with the health effects sections except for hematological and testicular toxicity and cancer. In the case of hematological toxicity, the mechanism involving methemoglobin formation is pretty well settled. Potential mechanisms for testicular effects and cancer are adequately covered in the profile. I do not have any additional citations regarding mechanisms to suggest.

RESPONSE: *No response needed.*

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: The conclusions given in this chapter at various points appear well supported by the information presented and overall database. I have no suggested changes or alternative conclusions to propose.

RESPONSE: *No response needed.*

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: The sections on absorption, distribution, metabolism, and excretion are very well done and thorough. The linkage of the metabolism discussion with potential modes of action for adverse nitrobenzene health effects is an important aspect of this section. I have no suggestions for improvement.

RESPONSE: *No response needed.*

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

COMMENT: The BPBK model of Guy (1985) is presented in the profile. It is, to my knowledge, the only model available for nitrobenzene.

RESPONSE: *No response needed.*

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: Nitrobenzene toxicokinetics observed in humans and animals are initially presented in the discussion of individual studies. Section 3.1.6 Animal-to-Human Extrapolations follows, and highlights toxicokinetic differences between animals and humans. Collectively, this information provides an adequate discussion of the comparative toxicokinetics of nitrobenzene among species.

RESPONSE: *No response needed.*

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: I'm not aware of any relevant data regarding child health and developmental effect that are not discussed.

RESPONSE: *No response needed.*

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: The discussion of populations at higher risk because of genetic susceptibility is well presented. The discussion of susceptibility due to co-morbidity is extremely brief and incomplete. Goldfrank et al. 1998) is cited as stating that individuals with pre-existing diseases (some examples are listed) may be at risk of nitrobenzene-induced methemoglobinemia at lower exposure levels. The citation is to the 6th edition; that book is currently in the 11th edition. The current edition unfortunately does not

mention nitrobenzene specifically as a cause of methemoglobinemia, but does offer other examples, including drugs (see Chapter 124). The chapter author also makes the point that susceptibility can be created by multiple stressors, stating that underlying illness, the treatment with xenobiotics for these illnesses, and the diagnostics and therapeutic modalities in patient care all contribute to predispose individuals to methemoglobinemia. Several citations are included for this statement (see page 1705). I suggest expanding this section on at-risk populations to include not only individuals with pre-existing diseases, but also individuals taking methemoglobin inducing drugs and co-exposure to other methemoglobin-inducing xenobiotics, providing examples and citing additional and newer references.

RESPONSE: *An additional paragraph has been added to Section 3.2 (Children and Other Populations That are Unusually Susceptible) outlining additional data on methemoglobin inducing groups and other xenobiotics.*

The paragraph reads "In addition, external factors such as medications and exposure to xenobiotics from the environment can also cause methemoglobinemia. Nitrite-based medications which are widely used to treat angina and other cardiac related problems can cause methemoglobinemia and are reported as a complication of the therapeutic use of these drugs (Bojar et al., 1987; Marshall, 1980). Self-administration of local anesthetic drugs like benzocaine have also been known to cause this condition (Nappe, Pacelli, & Katz, 2015).

Dapsone, a commonly used anti-inflammatory for treating infections, has severe side effects including methemoglobinemia, and patients are often recommended to use pulse oximeter to monitor blood oxygen levels regularly (John V. Ashurst, 2010; Mahmood, Khan, Haq, Jelani, & Tariq, 2019; Toker, Yesilaras, Tur, & Toktas, 2015). Acquired methemoglobinemia can also be caused by malaria medication (Kudale, 2014)."

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

COMMENT: Urinary metabolites of nitrobenzene could indicate exposure but are not specific to nitrobenzene. The profile does not recommend using them as biomarkers of exposure. I agree.

RESPONSE: *No response needed.*

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

COMMENT: Nitrobenzene increases methemoglobin, and methemoglobin levels in blood could be used as a biomarker for effect. However, as the profile points out, there are many substances that produce methemoglobin, and this effect is consequently not specific to the substance. The profile also discusses measurement of aniline in the blood from breakdown of nitrobenzene metabolite hemoglobin adducts as a more specific biomarker for nitrobenzene-induced methemoglobinemia. Possible limitations in this approach are noted.

RESPONSE: *No response needed.*

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: The discussion of potential interactive effects with other chemicals is limited, no doubt because there are few studies in the literature on this topic. I'm not aware of any study that explicitly demonstrates it, but it is reasonable to expect that other substances that increase methemoglobin could interact with nitrobenzene to produce an exaggerated effect. This could conceivably include chemicals found at hazardous waste sites such as nitrates or nitrites. This may be worth mentioning in this section.

RESPONSE: *A search of the literature was conducted to determine if there were any articles on the topic of interactive effects of nitrobenzene and other chemicals which increase methemoglobin, and no such article was located. However, it is theoretically possible that exposure to multiple chemicals which operate through the same mechanism of action would have some sort of additive or synergistic effect. Therefore, the following sentence was added to section 3.4 (interactions with other chemicals) "In addition, there are several other chemicals which operate through a similar mechanism of action in causing increases in methemoglobin such as nitrates and nitrites. Exposure to multiple methemoglobin inducing agents would likely increase the risk of an adverse outcome."*

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: The section is brief and mechanisms are not discussed, except to say that increased nitrobenzene toxicity with alcohol cotreatment does not appear to be due to increased absorption. Available information on interactions is so limited that there really isn't much to discuss in terms of mechanisms in this section.

RESPONSE: *No response needed.*

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 anything in the tables to be wrong or missing.

RESPONSE: *No response needed.*

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

COMMENT: Although nitrobenzene can exist either in crystalline form or as an oily liquid, there are not various forms of nitrobenzene. This question does not appear to be applicable to the subject of this profile.

RESPONSE: *No response needed.*

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: This information appears to be complete. I have no additional information to add.

RESPONSE: *No response needed.*

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: The text identifies potential points of release to the environment and explains likely pathways of exposure. The extent of occurrence of nitrobenzene at NPL sites is clearly shown in Figure 5-1 and associated text. I am not aware of any additional information that should be included.

RESPONSE: *No response needed.*

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: Transport, partitioning, transformation, and degradation in all relevant media are described in the text. I am not aware of any additional information that should be included on these topics.

RESPONSE: *No response needed.*

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: Information on occurrence of nitrobenzene in the environment, including background levels, is included in this section. Concentration units are appropriate to the medium. As nitrobenzene does not occur in multiple forms, this is not an issue when presenting concentration data. The profile includes caveats regarding potential sources of information such as the TRI, which is very helpful for the reader to understand limitations in the data. I have no additional information to add regarding environmental monitoring.

RESPONSE: *No response needed.*

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: There is a section devoted to sources and exposure pathways for the general population and workers. Populations with potentially high exposures are specifically addressed. I agree with the populations selected and do not have other populations to include.

RESPONSE: *No response needed.*

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 am not aware of any other studies that would fill a data gap.

RESPONSE: *No response needed.*

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

COMMENT: I agree with the identified data gaps. They flow logically from the information presented in the preceding sections of the document. The only gap I would suggest adding is the development of a PBPK/PD model for nitrobenzene to reduce uncertainty in extrapolating exposures from animal studies to humans.

RESPONSE: *The following sentence was added to section 6.2 (identification of data gaps) in the sub-section on comparative toxicokinetics "In addition, the development of a PBPK/PD model for nitrobenzene would also be useful, in order to reduce the uncertainty in extrapolating dose and effect information from animals to humans."*

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

COMMENT: Data needs are presented in a neutral fashion – I did not detect any bias.

RESPONSE: *No response needed.*

Chapter 7. Regulations and Guidelines

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

COMMENT: I'm not sure why Table 7-1 shows "no data" for EPA RfD and RfC. The EPA has an oral RfD and an inhalation RfC listed on IRIS for nitrobenzene.

RESPONSE: *The RfD and RfC in Table 7-1 were updated to the values in the IRIS, which are 2 x 10^{-3} mg/kg-day and 9 x 10^{-3} mg/m^3, respectively.*

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

COMMENT: I don't see any that should be removed.

RESPONSE: *No response needed.*

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: MRLs are derived except for acute and chronic oral exposure. I agree that the data do not support deriving an acute or chronic oral MRL.

RESPONSE: *No response needed.*

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.

a. 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: Acute Inhalation MRL – A UF of 90 is used based upon individual UFs of 10, 3, and 3. By convention, a UF of 3 is usually considered a half-log, and two combined would be 10 instead of 9. More importantly, the second factor of 3 is presented as a modifying factor based upon "the BMCL1SD being based on methemoglobin levels, and that there are several differences in rodent versus human physiology in this endpoint." Rodent versus human physiological differences are supposed to be accounted for in the animal to human extrapolation factor, which was the other factor of 3. It appears to me that the UF should be 30 (10 for intrahuman variability and 3 for extrapolation from animal to humans after dosimetric adjustment).

Intermediate Inhalation MRL – No comments or suggestions. I agree with each component of the total UF selected.

Chronic Inhalation MRL – Because different methods are being used to calculate human equivalent doses for inhalation exposure in Appendix A, some additional discussion is needed regarding the selection of the regional gas dose ratio for chronic inhalation of nitrobenzene. I agree with each component of the total UF selected.

Intermediate Oral MRL – The key issue on this one is using the non-constant variance to derive a benchmark dose model with an acceptable fit. Otherwise, a switch to a different endpoint or NOAEL/LOAEL approach would be needed. The profile states, 'Using the NCV assumes that "the variance changes as a power function of the mean value.' To test this assumption we plotted the mean values against the variance. The R2 value for the fit of the data to a power function trend line in Excel was 0.7, which we assumed was sufficient to assume NCV." It might strengthen the case to show plot and provide some stronger backup that an R2 of 0.7 is good enough. I agree with each component of the total UF selected.

RESPONSE: *Regarding the acute inhalation MRL ATSDR has not changed the UF from 90 to 30 as suggested by the peer reviewer. The UF of 3 for animal to human extrapolation account for known differences in the toxicokinetic and dynamic differences between the rat and the human. However, there are distinctive metabolic differences between rats and humans which are not accounted for in the default UF of 3 for animal to human extrapolation. It is well documented that humans have much less methemoglobin reductase compared to rodents. To account for this known additional susceptibility of adversity when comparing humans to animals ATSDR has determined that the MF of 3 is needed. Further, given the MF applied is 3 (and not the half-log of 10) the UF remains 90 and is not changed to 100.*

For the comment on the chronic inhalation MRL, additional details were added to Appendix A to explain that the application of the RGDR is following U.S. EPA guidance. Specifically, the section on the Human Equivalent Concentration for the chronic inhalation MRL now reads "Given the critical effect occurs in the respiratory system and is not a systemic effect a regional gas dose ratio (RGDR) is needed to estimate a $BMCL_{HEC}$ *(as opposed to the blood:air partition coefficient). Further the effects are due to the distribution of nitrobenzene through the extra thoracic region (as opposed to the pulmonary region or a systemic effect). Following EPA's guidance (U.S. EPA 1994) we used the following equation:…"*

For the comment on the Oral MRL regarding the Non-Constant Variance (NCV), for clarity we removed the text referencing the R^2 *value. To clarify and support the assumption of NCV the following text was added to Appendix A for the intermediate ORL MRL. Specifically, in the Selection of the Point of Departure for the (provisional) MRL reads a new paragraph reads: "However, running the modeling using a nonconstant variance (NCV) did provide suitable model fits and tests on the assumption of NCV confirmed the assumption of NCV. Specifically, Test 2 in the BMDS output, which tests the null hypothesis that variances are homogenous was significant (P =0.012), indicating that non-constant variance is appropriate. Further, Test 3, which tests the null hypothesis that the variances are adequately modeled (A3 vs A2). Had a p-value > 0.01 inferring that the variances have been modeled appropriately."*

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

COMMENT: I have no additional comments on the MRL database assessment.

Minor editorial comment: I may have missed it, but there should be a call-out in text for Figures 1-3 and 1-4. Also, a footnote should be provided for these figures to indicate what specifically the numbers in the figure represent (e.g., LOAEL for the effect).

RESPONSE: *Text has been added to section 1.3 (Minimum Risk Levels) to call out these figures. The text reads "As illustrated in Figure 1-3, hematological, hepatic, renal, respiratory and endocrine effects appear to be the most sensitive targets of nitrobenzene inhalation. Hematological, hepatic, and cardiovascular effects appear to be the most sensitive targets of ingested nitrobenzene (Figure 1-4). The lowest-observed-adverse effect levels (LOAELs) in Figures 1-3 and 1-4 reflect actual doses (levels of exposure) employed in animal studies."*

Appendices

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

COMMENT: Appendix A provides the technical support for derivation of the MRLs. Organization and presentation of information is consistent with the format used in other recent toxicological profiles. This presentation provides a clear description of the information considered most relevant for MRL development, as well as the rationale for key decisions in regarding principal study, critical effect, dose-response assessment, and incorporation of uncertainty factors. Comments on specific MRLs are provided above.

Appendices B-E provide information on the literature search for nitrobenzene, as well as generic information for health care providers, a user's guide, and glossary. These sections are generally well written and presented and I have no comments or suggestions.

RESPONSE: *No response needed.*

Unpublished Studies (Applicable for this Review)

Comments regarding the unpublished studies compiled from separate documents sent by peer reviewers:

Chemfirst (1998). Toxicity testing of nitrobenzene for the first chemical corporation with cover letter dated 7/24/1998 (sanitized).

Reviewer #1

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: The study was conducted under a standard protocol, and the number of animals was adequate for the purposes of the study. Details regarding animal care are not included in the brief report but there is nothing in the report that is concerning**.**

QUESTION Did the study account for competing causes of death?

COMMENT: The lethality study did not include a control group, which is typical for studies of this type. However, mortality would not be expected from other causes in an acute study in young animals such as this**.**

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: The study conducted was a limit test to determine whether the compound could be considered "non-toxic" (i.e., LD50 > 2 g/kg). For that purpose, the number of dose groups and magnitude of dose levels were adequate.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study design and reporting were adequate for the purpose of the study**.**

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: I agree with the authors' conclusions regarding dermal and ocular irritation, as well as the median lethal dose in rabbits.

RESPONSE: *While this reviewer did not point out issues in the study, as a result of the other 2 peer reviewer's comments pointing out flaws, the reference, Chemfirst. 1998. Toxicity testing of nitrobenzene*

for the first chemical corporation with cover letter dated 7/24/1998 (sanitized), and all reference to this article were removed from the profile.

Dupont (1981). Initial Submission: Acute Inhalation Toxicity Of Benzene, Nitro In Male Crl:Cd Rats With Cover Letter Dated 090292.

Reviewer #1

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: The number of animals is adequate, and consistent with methods used at the time. Newer approaches use fewer animals for an acute lethality study, but the approach used then would still be considered valid. Details regarding animal care are not included in the brief report but there is nothing in the report that is concerning.

QUESTION: Did the study account for competing causes of death?

COMMENT: The study design does not include a control group, which is typical for studies of this type. However, mortality would not be expected from other causes in an acute study in young animals such as this**.**

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: The number of dose groups and magnitude of dose levels were sufficient to determine an acutely lethal concentration with good confidence.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study design was adequate for the purpose and the report, although brief, was sufficient to provide the minimum necessary information about the study and its results.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: The report principally provided observations regarding mortality rates and clinical observations in animals given an acute, high inhalation exposure to nitrobenzene. The author(s) provide no conclusions.

RESPONSE: *The Dupont study was retained in the profile as the peer reviewers identified that the study provides relevant information.*

Annotated Comments on the Profile

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

Reviewer #2 did not provide point by point answers to the charge questions. However, they provided one overall comment for each set of questions on a chapter. ATSDR responded to these overarching comments as needed.

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.

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.

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

COMMENT: The effects of nitrobenzene in humans are appropriately listed as are the major routes of exposure. The animal model studies well support the largely anecdotal human data with methemoglobinemia being one of the main effects of nitrobenzene in both animals and humans. The relevance of the animal effects are well discussed with relevance to humans with appropriate discussion of the exposure conditions. The minimum risk levels are well presented both in the figures and appendix A.

RESPONSE: *No response needed*

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.

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.

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.

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?

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

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.

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.

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.

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.

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.

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: This chapter provides an adequate coverage of the literature regarding the health effects of nitrobenzene exposure. The works cited cover the exposure routes most commonly occurring namely, inhalation, ingestion, and dermal. All major organ systems are covered and there is good discussion of the relative doses of exposure between human and animal studies. The majority of the human data concerns case report information, however, these studies confirm the applicability of the animal data generated. The animal models (rats, mice, and rabbits) are appropriate toxicological models and the potential complications, such as differential levels of methemoglobin reductase, have been considered.

I am not aware of specific gaps in the literature cited and the calculations for MRL, NOAEL, and LOAEL are appropriate with direct reference to the primary literature values. Effects have been appropriately categorized as serious and less serious and there is appropriate consideration of mechanisms. The mechanisms given are appropriate at the pathophysiological level most notably the consideration of methemoglobin formation and its effects as a form of hemolytic anemia. The specific chemical mechanisms involved in nitrobenzene mediated iron oxidation are given in chapter 3. The conclusions of this chapter are appropriate

RESPONSE: *No response needed.*

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.

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

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?

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.

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.

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

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

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.

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: This chapter contains significant and relevant information on the ADME of nitrobenzene. Appropriate comparisons are made between human and animal data notwithstanding the small amount of human data available. In conjunction there are only limited PBPK models available, however, these are well discussed and delineated. The potentially significant difference in species expression of metHb reductase is included and is well discussed.

Susceptible populations are noted as being children, especially neonates, due to their low expression of metHb reductase and the presence of fetalHb. In addition, the possibility of those with Hb anomalies, as well as other metabolic deficiencies such as GAPDH deficiency, being susceptible is well considered.

As nitrobenzene is generally converted by either reduction or oxidation it is not a significant element for biomarker measurement. However, there are significant metabolites that can be used although these may also derive form other sources. This is well discussed. The appropriateness of metHb as a biomarker of effect is well given. There is also significant discussion of potential interacting chemicals and the possibility of combined oxidative stress.

RESPONSE: *No response needed.*

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.

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

COMMENT: The chemical and physical information given in this chapter appears to be correct and sufficient information on both the crystallized and solution form of nitrobenzene are given.

RESPONSE: *No response needed.*

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.

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.

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.

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.

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: This chapter provides complete information on the production, use, and disposal of nitrobenzene. There is sufficient description of the tracing of nitrobenzene from its production, either industrial or through atmospheric generation, to the points of human exposure. Correctly the report discusses atmospheric and terrestrial release mechanisms and points out that the majority of human exposure is likely to come from workplace or being in vicinity of a contaminated site. There is good information on levels that have been monitored and the media in which these levels have been measured. Appropriate descriptions are given for populations that may be exposed.

RESPONSE: *No response needed*

Chapter 6. Adequacy of the Database

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

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

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

COMMENT: This chapter provides a well written discussion of the adequacy of the database and points out the areas that need improvement such as acute oral MRL. It takes into consideration the data form both human and animal studies and considers sensitive populations such as children.

RESPONSE: *No response needed*

Chapter 7. Regulations and Guidelines

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

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

COMMENT: All the appropriate regulations appear to have been reported and considered.

RESPONSE: *No response needed.*

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.

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.

a. 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.

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

COMMENT: No comments provided.

RESPONSE: *No response needed.*

Appendices

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

COMMENT: These are satisfactory

RESPONSE: *No response needed*

Unpublished Studies (Applicable for this Review)

Comments regarding the unpublished studies compiled from separate documents sent by peer reviewers:

Chemfirst (1998). Toxicity testing of nitrobenzene for the first chemical corporation with cover letter dated 7/24/1998 (sanitized).

Reviewer #2

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: The study does use a sufficient number of animals although a prior power calculations are not provided. However, the significant problem with this study is that it would not be considered good

animal care by current research standards. For appropriate studies under today's guidelines symptoms of severe morbidity should be utilized for determination of lethality and animals sacrificed before death. In addition, sex was not considered as a variable within the rabbit studies.

QUESTION: Did the study account for competing causes of death?

COMMENT: The pathology appears to have been performed adequately, however, histology is not shown.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: The doses for oral lethality in rabbit were too close and too high to determine an LD50 and are thus inadequate.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: I do not think the study was fully well-designed and this to some extent limits the applicability of the data.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: I agree with the rat oral LD50, that the rabbit LD50 is greater than 2g/kg, and that at the doses used nitrobenzene was not an irritant.

RESPONSE: *As a result of the peer reviewer's comments regarding the dose levels, the comments of peer reviewer 3 identifying flaws, the article Chemfirst. 1998. Toxicity testing of nitrobenzene for the first chemical corporation with cover letter dated 7/24/1998 (sanitized), and all reference to this article were removed from the profile.*

Dupont (1981). Initial Submission: Acute Inhalation Toxicity Of Benzene, Nitro In Male Crl:Cd Rats With Cover Letter Dated 090292.

Reviewer #2

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: The study does use a sufficient number of animals; however, this study would not be considered good animal care by current research standards. For appropriate studies under today's guidelines symptoms of severe morbidity should be utilized for determination of lethality and animals sacrificed before death. In addition, sex was not considered as a variable within the rabbit studies.

QUESTION: Did the study account for competing causes of death?

COMMENT: The lethality is appropriately reported however no histology is given and clinical signs are given.However, pathology and necropsy were not performed and thus cause of death cannot be stated.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: The dose range is narrow but does appear to span the LD50

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: No response provided.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: The conclusion of an inhalation LD50 for rate in a 4 hour exposure of 556 ppm appears correct. This data while compromised is reasonable in assessing nitrobenzene lethality

RESPONSE: *The Dupont study was retained in the profile as the peer reviewers identified that the study provides relevant information.*

Annotated Comments on the Profile

COMMENT: Should add potential bone marrow suppression following ingestion. Also suggested the citation of Burns 1994, Immunotoxicity of nitrobenzene in female B6C3F1 mice.

RESPONSE: *The reviewer suggests adding this citation and text to the hematological bullet in section 1.2 (Summary of Health Effects). The reviewer left this comment on the following sentence: "A variety of other adverse effects in the hematologic system such as extramedullary hematopoiesis, alterations in hemoglobin levels, and congestion of the spleen have also been observed with inhalation, oral and dermal exposures in B6C3F1 mice, F344 rats, and Sprague-Dawley rats (Cattley et al. 1994; Hamm Jr. et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b). Upon review of the suggested article "Burns et al. 1994. Immunotoxicity of Nitrobenzene in Female B6C3F1 Mice. Drug and Chemical Toxicology. 17(3): 271-315" it became apparent this study was not only appropriate to include in the suggested sentence, but it should be incorporated throughout the profile. Additional text in the profile was added to the following sections, with the added text accompanying each bullet:*

- *Section 2.2 (Death): "In an acute-duration oral toxicological evaluation in female B6C3F1 mice 8.5% of the mice given 300 mg/kg nitrobenzene in corn oil for 14 days died (Burns et al., 1994)."*
- *Section 2.3 (Body Weight): "Female B6C3F1 mice which were administered nitrobenzene for 14 days via gavage with corn oil displayed a 12% increase in body weight (Burns et al., 1994). The authors hypothesized this increase in body weight was likely due to fluid retention in the high dose group. Female mice receiving 100 mg/kg in the same study did not demonstrate a significant increase in body weight (Burns et al., 1994)."*
- *Section 2.4 (Respiratory): "In Burns et al., (1994) researchers observed a significant increase in absolute lung weight with 30 mg/kg nitrobenzene exposure in B6C3F1 mice gavaged with nitrobenzene for 14 days. However, the same increase was not observed considering relative lung weight. Therefore, the authors hypothesized that the mechanism leading to fluid retention which was considered the culprit for increasing body weight may have also contributed to the increases in lung weight (Burns et al. 1994)."*
- *Section 2.7 (Hematological): "In an acute-duration oral exposure study B6C3F1 female mice were exposed to nitrobenzene via corn oil gavage for 14-day (Burns et al., 1994) at doses of 30, 100 and 300 mg/kg. In this study the most sensitive effect observed with nitrobenzene exposure were perturbations in the bone marrow where the number of cells in the femur bone marrow, DNA synthesis, and the number of colony forming units for granulocyte monocyte progenitor cells were increased in a statistically significant manner following a dose-response trend, starting at 30 mg/kg/day. In addition, Burns et al. (1994) observed results consistent with nitrobenzene's hematological toxicity. Specifically, the number of erythrocytes were decreased*

with compensatory increases in mean corpuscular volume and mean corpuscular hemoglobin, starting at doses of 100 mg/kg. In addition, the percentage of reticulocytes increased dose-dependently, also starting at doses of 100 mg/kg. Hepatomegaly and splenomegaly were observed by the researchers as were pathological observations consistent with extramedullary hematopoiesis."

- *Section 2.9 (Hepatic): "In Burns et al. (1994) relative liver weight increased dose-dependently starting at 100 mg/kg in B6C3F1 female mice dosed for 14 days. In addition, ALT was significantly increased with 300 mg/kg exposure in the same study. Further, at 300 mg/kg the liver displayed minor histopathological changes including mild hydropic degeneration around focal central veins"*
- *Section 2.10 (Renal): "In an acute duration exposure study B6C3F1 mice exposed to nitrobenzene for 14 days via gavage experienced a 10% increase in absolute but note relative kidney weight with 300 mg/kg exposure. There was not a significant increase in kidney weight at 100 mg/kg (Burns et al., 1994)."*
- *Section 2.14 (Immunological): "In an acute duration oral exposure study, B6C3F1 female mice were administered 0, 30, 100 or 300 mg/kg of nitrobenzene in corn oil via gavage (Burns et al., 1994). In this study Burns et al. (1994) observed immunotoxicity with nitrobenzene exposure including decreases in IgM response in the spleen to T-dependent antigens, alterations in phagocytic activity of macrophages and decreased activity of natural killer cells starting at 100 mg/kg exposure. Host resistance to microbial infection was not impacted by exposure to nitrobenzene in this study. However, there was a trend toward increased susceptibility of the mouse when the host defense is dependent on T-cell functioning. In addition, alterations in bone marrow activity were observed including increases in the number of colony forming units for granulocyte monocyte progenitor cells were increased in a statistically significant manner following a dose-response trend, starting at 30 mg/kg/day (Burns et al., 1994)."*
- *Section 2.15 (Neurological): "In an acute duration oral exposure study brain relative brain weight decreased in B6C3F1 mice exposed to nitrobenzene via gavage at doses of 300 mg/kg. The same difference did not occur with 100 mg/kg exposure (Burns et al., 1994)."*

In addition, the Burns et al. 1994 paper also had information to inform an acute-duration oral MRL. ATSDR utilized benchmark dose modeling for data on DNA synthesis in the bone marrow and conducted internal and interagency review. The reviews concluded the new MRL was appropriate and has thus been incorporated in the profile.

COMMENT: Does is given but not time period. Whats the total exposure?

RESPONSE: *This comment refers to the following sentence in the second paragraph in section 2.3 (Body Weight): "In an acute inhalation study on the toxicity of nitrobenzene in pregnant New Zealand white rabbits, nitrobenzene was administered via inhalation at concentrations of 10, 40, and 80 ppm." The text "for six hours per day" was added to this sentence for clarity. This information is also presented in the LSE table.*

COMMENT: 24 hours a day? Please give the exposure time of the study

RESPONSE: *This comment refers to the following sentence in section 2.3 (Body Weight): "In a study investigating chronically inhaled nitrobenzene in B6C3F1 mice and Fischer 344 and Sprague-Dawley rats, neither mice nor rats exposed to 50 ppm nitrobenzene for 90 days exhibited reductions in body weight (Cattley et al. 1994)." The text "for six hours per day" was added to this sentence for clarity. This information is also presented in the LSE table.*

COMMENT: Time period of exposure

RESPONSE: *This comment pertains to the following statement in section 2.4 (Respiratory): In an acute, 2 week inhalation study of nitrobenzene toxicity on male and female F344 and Sprague-Dawley (CD) rats and B6C3F1 mice (Medinsky and Irons 1985) researchers observed 8/10 mice with moderate bronchiolar epithelial hyperplasia after 125 ppm nitrobenzene exposure and hyperplasia presenting in animals examined 3 days after an exposure of 35 ppm. The text "for six hours per day" was added to this sentence for clarity. This information is also presented in the LSE table.*

COMMENT: This article could be added here:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916109/

RESPONSE: *The reviewer suggests adding this article, the citation for which is Lee CH, Kim SH, Kwon DH, et al. 2013. Two cases of methemoglobinemia induced by the exposure to nitrobenzene and aniline. Annals Of Occupational And Environmental Medicine 25(1):31-31 to the second paragraph in section 2.7 (Hematological). The citation suggested is already cited in the flagged sentence and therefore no edits have been made.*

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: Yes. N/a, although I did note in comments in the document at least one reference that is missing from the reference list.

RESPONSE: *The peer reviewer pointed out that the NTP citation was missing in the reference list. The reference list has been checked, and NTP 1982 is included: NTP. 1982. Repeated dose dermal toxicity test of nitrobenzene in fischer 344 rats and B6C3F1 mice. Research Triangle Park, NC: Prepared by the EG&G Mason Research Institute Prepared by the EG&G Mason Research Institute, Worcester, MA, for the National Toxicology Program, National Institute of Environmental Health Services, Public Health Service, U.S. Department of Health and Human Services MRI-NTP 17-82-28.*

Other comments on missing references are addressed in the annotated comment and response.

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 does not seem that there are a lot of exposed humans who were enrolled in medical surveillance programs, so I don't think that we can discount them as totally irrelevant to humans.

RESPONSE: *No response needed.*

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

COMMENT: Yes

RESPONSE: *No response needed.*

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: No changes suggested

RESPONSE: *No response needed.*

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: It seems that a lot of the human exposures were frank poisonings. I am not sure that that caveat was emphasized, although the mention of the studies occurred multiple times in the document.

RESPONSE: *Additional caveats were added throughout the profile to clarify that the amount of exposure is not known in most of the case studies and reports of poisonings.*

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: Without reading the studies, I cannot comment on aminal care. Most of these studies were performed at contract research labs with appropriate numbers of animals. I do have concerns about the First Chemical Corporation document which I will share in a separate document. I do wonder if the interpretation of the reproductive toxicity study would be the same if interpreted by today's standards.

RESPONSE: *Given the comments on the study by Chem First for the First Chemical corporation the data from this study has been removed from the profile.*

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: No concern here.

RESPONSE: *No response needed.*

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

COMMENT: Not a lot of dose-response for human exposures.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: This is one of many instances of where ATSDR uses language that is not consistent with toxicology terms. Toxicologists are generally interested in the toxic effect that occurs at the lowest level of exposure in a study.

RESPONSE: *As noted in the profile "ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear." Subsequently no edits have been made in response to this comment.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: Nothing to add, except that it's hard to believe that someone has not identified the CYP enzymes responsible for the oxidative biotransformation of nitrobenzene. I searched for these data but could not find any**.**

RESPONSE: *No response needed.*

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

COMMENT: Nothing to add.

RESPONSE: *No response needed.*

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: Data in this section are quite old, and do not address magnitudes of exposure when making statements such as "Humans have slower metabolism……..by an order of magnitude……. Were the exposure levels equivalent when coming to this conclusion?

RESPONSE: *Although the data in the section are old, they are still correct; more recently published data were not located during the development of the profile. Further, given the lack of citation for the sentence in question it was removed from the profile.*

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 meaningful discussion was found of childhood exposure, except that metabolites were found in the NHANES data, but there was no discussion of effects in human children. Developmental effects were discussed in two laboratory animal studies.

RESPONSE: *In developing the profile ATSDR reviewed the body of literature on nitrobenzene and did not locate information specific to children being exposed. Therefore, the only data available to summarize were those on laboratory animals as noted by the reviewer. Subsequently no revisions were made in response to this comment.*

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: I found this section to be quite good.

RESPONSE: *No response needed.*

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

COMMENT: No specific biomarker of exposure, except for nitrobenzene in blood, reflecting recent exposure.

RESPONSE: *No response needed.*

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

COMMENT: There are limited exposures that cause methemoglobinemia in humans, so with a history of exposure to nitrobenzene, methemoglobinemia is probably a pretty good biomarker of effect.

RESPONSE: *No response needed.*

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: This section cites very old studies and I'm not sure that death as an endpoint is a good endpoint for drawing conclusions about effects that might occur at hazardous waste sites.

RESPONSE: *The studies cited in Section 3.4 (Interactions with Other Chemicals) were the only available studies which directly evaluated interactions with other chemicals that were located in the process of developing the profile. Based on comments from another reviewer text was added to the interaction section stating "there are several other chemicals which operate through a similar mechanism of action in causing increases in methemoglobin such as nitrates and nitrites. Exposure to multiple methemoglobin inducing agents would likely increase the risk of an adverse outcome." Given the lack of data to update the text in this section no changes have been made in response to this comment.*

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 discussion of mechanism is presented.

RESPONSE: *Given the paucity of data on interactions of nitrobenzene with other chemicals we are not able to present a mechanism by which an interaction between the chemicals may occur. As previously noted based on comments from another reviewer text was added to the interaction section stating "there are several other chemicals which operate through a similar mechanism of action in causing increases in methemoglobin such as nitrates and nitrites. Exposure to multiple methemoglobin inducing agents would likely increase the risk of an adverse outcome." No other edits were made in response to this comment.*

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 verify these values in the cited sources.

RESPONSE: *No response needed.*

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

COMMENT: I am not aware of multiple forms on nitrobenzene.

RESPONSE: *No response needed.*

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: Nothing to add; seems complete.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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: This section is quite brief, but I am not aware of any other exposure data.

RESPONSE: *No response needed.*

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: Nothing to add.

RESPONSE: *No response needed.*

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

COMMENT: Given limited exposure to nitrobenzene by oral exposure, I am not sure that resources should be used to perform a study to derive an oral MRL. Endocrine effects in worker populations might be useful if exposures are not kept to a minimum with PPE and engineering controls. I'm not knowledgeable about respirators, ventilation, etc., that are used to minimize nitrobenzene exposures. I'm not sure about the statement that fetal malformations in rodents should be further studied, given how large the exposures were in these rodent studies. For cancer, again, I don't think that focus on oral exposures is warranted.

RESPONSE: *Given there is insufficient data to derive MRLs for all durations of exposure for nitrobenzene, and there is still a potential for oral exposure (albeit the potential is lower than inhalation) there is still a gap in this data. Further, given that there is some uncertainty regarding the fetal malformations ATSDR did not remove the statement that further studying the presence of malformations after nitrobenzene exposure would be beneficial, as additional studies may decrease the uncertainty. Subsequently no edits were made in response to this comment.*

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

COMMENT: I suggested removal of some subjective language in appendix a, including pp A-18 and A-19.

RESPONSE: *The reviewer suggested removing the works "just" and "only" when preceding doses of 1 and 5 ppm in Appendix A in reference to the doses given in the chronic inhalation study used for MRL. These words were removed as suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: Nothing to add.

RESPONSE: *No response needed.*

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

COMMENT: Nothing to add.

RESPONSE: *No response needed.*

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: *No comment was provided by the reviewer.*

RESPONSE: *No response needed.*

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.

a. 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 comment was provided by the reviewer.*

RESPONSE: *No response needed.*

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

COMMENT: I am not a modeler, but I carefully reviewed the Appendix and find that the calculations and assumptions seem reasonable.

RESPONSE: *No response needed.*

Appendices

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

COMMENT: Please see separate documents.

Unpublished Studies (Applicable for this Review)

Comments regarding the unpublished studies compiled from separate documents sent by peer reviewers:

Chemfirst (1998). Toxicity testing of nitrobenzene for the first chemical corporation with cover letter dated 7/24/1998 (sanitized).

Reviewer #3

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Study used minimum number of animals, per guidelines included in the report. I am not able to assess animal care practices.

QUESTION: Did the study account for competing causes of death?

COMMENT: I did not see any explanation of causes of death in the report.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Rabbit dermal irritation study used a single dose of 0.5 cc, which appears to be acceptable per the guidelines provided in the report. For the oral lethality study, the rationale for the dose range (246-399 mg/kg) and the dose spacing (246, 290, 333, 348, 355, 362, 399 mg/kg) is not clear. I am struggling with the reporting of the data, which might be in Table 3 (title does not indicate which study is summarized in the table), but a puzzling comment in the legend to the table refers to "Lethal doses above 275". 275 is not a dose in the study. In the dermal lethality study, there appears to be a dosing error (animal 63) and an error in reporting (rabbit 64 is reported to have died on 2/4/85, which is prior to the starting day of the study (2/19/85). Table 1 indicates animal ID numbers for the rabbits in the eye irritation study, but it does not appear that any data are entered into the table.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Given the issues mentioned above for the oral and dermal lethality study, I don't have a lot of confidence in the results of those studies. In more carefully reading the study results, section III, authors indicate that Table 3 is dermal lethality, whereas table 4 is labeled as presenting dermal lethality data. Which must mean that Table 3 is actually the oral lethality data. Very careless reporting.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: In reading the authors' conclusions, confidence is eroded by erroneous references to the tables. I'm not sure that an LD50 could be calculated for the dermal lethality study, as only one dose was used, so there's no dose-response curve. For the oral LD50, the printouts to which the reader is pointed are incomprehensible, so I cannot say whether I agree or disagree with this LD50 value (349 mg/kg). Table 1 indicates animal ID numbers for the rabbits in the eye irritation study, but it does not appear that any data are entered into the table, so I cannot agree or disagree with their assessment that nitrobenzene is not an eye irritant.

RESPONSE: *As a result of the peer reviewer's comments, Chemfirst. 1998. Toxicity testing of nitrobenzene for the first chemical corporation with cover letter dated 7/24/1998 (sanitized) and all reference to this article were removed from the profile.*

Dupont (1981). Initial Submission: Acute Inhalation Toxicity Of Benzene, Nitro In Male Crl:Cd Rats With Cover Letter Dated 090292.

Reviewer #3

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Study used 8 male rats per test concentration, which seems adequate to me. Symptoms are reported in detail, so I am willing to believe that good animal care practices were in place. It's probably an artifact of when this study was done, but I find it odd that females were not also tested.

QUESTION: Did the study account for competing causes of death?

COMMENT: I did not see any explanation of causes of death in the report.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Exposure concentrations ranged from 439-714 ppm. The rationales for the concentration range and concentration spacing are not clear, but I believe that a sufficient number of concentrations were tested. Reporting, in terms of time to death and detailed accounts of symptoms, give confidence that this is a useful study, given the limited study design (single 4-hour exposure).

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: n/a

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: LC50 value of 556 ppm in male rats seems reasonable based on the data presented and the study design (a single 4-hour exposure).

RESPONSE: *The Dupont study was retained in the profile as the peer reviewers identified that the study provides relevant information.*

Annotated Comments on the Profile

COMMENT: This page, and the next page, are both numbered as page 1!

RESPONSE: *This comment refers to the page numbers in Chapter 1 (Relevance to Public Health). Page numbers have been updated.*

COMMENT: It would be so much easier on the reader if abbreviations were defined in the text, rather than making readers go to the glossary if they don't know what an abbreviation stands for.

RESPONSE: *This comment refers to the acronym "TRI" in section 1.1 (Overview and U.S. Exposures), and specifically the sentence that states "While most nitrobenzene is retained in closed loop systems, data collected for TRI suggests that 64,4532 pounds of nitrobenzene were released to the environment from industrial activities in 2017 (TRI17 2019)." The sentence has been edited to define TRI and now says "While most nitrobenzene is retained in closed loop systems, data collected for the Toxics Release*

Inventory (TRI) suggests that 64,4532 pounds of nitrobenzene were released to the environment from industrial activities in 2017 (TRI17 2019)."

COMMENT: Not a scientific term at all. I saw this term throughout the document but I will not flag it each time. Should be replaced with "significant" or some other scientific term.

RESPONSE: *This comment refers to the use of "stark" in the respiratory bullet in section 1.2 (Summary of Health Effects). The text stated "In the same study a stark increase in bronchiolization of the alveoli was observed in mice (Cattley et al. 1994). The word "stark" has been replaced with the word "significant."*

COMMENT: Again, I'm not going to flag this every time it occurs, but studies don't observe anything. Studies show/demonstrate, etc, but certainly not observe.

RESPONSE: *This comment refers to the use of "observed" in the following sentence from the respiratory bullet in section 1.2 (Summary of Health Effects): "In addition, acute and intermediate dermal exposure studies have observed lung congestion after nitrobenzene exposure in F344 rats (NTP 1982)." The phrasing in question was changed to "In addition, acute (≤14 days) and intermediate (15-364 days) dermal exposure studies have demonstrated lung congestion after nitrobenzene exposure in F344 rats"*

COMMENT: Missing from reference list

RESPONSE: *This comment refers to the NTP 1982 citation in the respiratory bullet in section 1.2 (Summary of Health Effects). The reference list has been checked, and NTP 1982 is included: NTP. 1982. Repeated dose dermal toxicity test of nitrobenzene in fischer 344 rats and B6C3F1 mice. Research Triangle Park, NC: Prepared by the EG&G Mason Research Institute Prepared by the EG&G Mason Research Institute, Worcester, MA, for the National Toxicology Program, National Institute of Environmental Health Services, Public Health Service, U.S. Department of Health and Human Services MRI-NTP 17-82-28.*

COMMENT: Again, not a scientific term. Subchronic would be more appropriate. Also, "intermediate" is used multiple times in this chapter, but not defined until chapter 2. The definition provided there is consistent with subchronic, and I suggest making that change throughout the document.

RESPONSE: *This comment refers to the use of "intermediate" in the hematological bullet in section 1.2 (Summary of Health Effects). The term is used in the following sentence: "Additionally, experimental animal studies have demonstrated an increase in methemoglobin levels in mice and rats of both sexes exposed through any exposure route with acute, intermediate and chronic exposure durations (Biodynamics 1984; Cattley et al. 1994; CIIT 1993; Hamm Jr. et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b)."*

ATSDR's definition of acute (≤14 days), intermediate (15-364) and chronic (≥365) are now defined at first use in chapter one. However, based on ATSDR's Guidance for the Preparation of Toxicological Profiles the terminology of "intermediate" duration exposure has not changed, as this is the standard language used by the Agency when referring to an exposure between 15 and 365 days.

COMMENT: BSP is a chemical probe for liver function. A word is missing, perhaps "clearance", to read "BSP clearance". Also, BSP is not included in the abbreviation list

RESPONSE: *This comment refers to the following sentence in the hepatic bullet in section 1.2 (Summary of Health Effects): "In the case studies there were disruptions in the liver as evidenced by a reduction in BSP and an increase in icterus index (i.e., jaundice) and indirect bilirubin levels in liver function tests (Ikeda and Kita 1964) and pathological observations of hepatic centrilobular necrosis (Gupta et al. 2012)." The sentence is questions was edited and now reads "In the case studies there were disruptions in the liver as evidenced by an increase in the retention of BSP (bromosulphthalein; a dye used in liver function test)…"Additionally BSP has been defined as bromosulphthalein in Appendix E.*

COMMENT: Incorrect usage again. "Observed" should be replaced with "presented with".

RESPONSE: *This comment refers to the use of "observed" in the hepatic bullet in section 1.2 (Summary of Health Effects). The term is used in the following sentence: " Experimental animal studies with inhalation and oral exposures observed a range of adverse liver effects, with the most common effects being necrosis and hepatocytomegaly in the centrilobular region (Cattley et al. 1994; Hamm Jr. et al. 1984; Medinsky and Irons 1985; NTP 1983a)." The text in question has been changed and now reads "Experimental animal studies with inhalation and oral exposures displayed a range of adverse liver effects."*

COMMENT: Change to "shown" or "demonstrated"

RESPONSE: *This comment refers to the use of "observed" in the renal bullet in section 1.2 (Summary of Health Effects). The term is used in the following sentence: "Several experimental animal studies have observed increases in kidney weight and degenerative changes in the cortical tubules (Medinsky and Irons 1985; NTP 1982, 1983a;)." The text in question has been changed and now reads "Several experimental animal studies have demonstrated increases in kidney weights"*

COMMENT: Extra space, or missing reference??

RESPONSE: *This comment refers to the intext citation "(Medinsky and Irons 1985; NTP 1982, 1983a;)" in the renal bullet in section 1.2 (Summary of Health Effects). There was an extra space, and it has been removed.*

COMMENT: Clumsy sentence—could be written more clearly.

RESPONSE: *This comment refers to the following in the reproductive bullet in section 1.2 (Summary of Health Effects): "Common effects seen after exposure to nitrobenzene include atrophy of the seminiferous tubules, hypospermatogensis, Sertoli cell hyperplasia and dysspermiogensis in F344 rats, B6C3F1 mice, Sprague-Dawley rats after dermal, inhalation and oral exposure of acute, intermediate and chronic duration (Cattley et al. 1994; Dodd et al. 1987; Hamm Jr. et al. 1984; Kawaguchi et al. 2004; Kawashima et al. 1995; Linder et al. 1992; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b)." The flagged text was rewritten to now read "Common effects seen after exposure to nitrobenzene include atrophy of the seminiferous tubules, hypospermatogensis, Sertoli cell hyperplasia and dysspermiogensis. These effects have been demonstrated in a variety of rodent species after acute, intermediate and chronic duration exposure via all exposure routes"*

COMMENT: I suggest defining MRL prior to referring the reader to Figs 1-1 and 1-2. Also, suggest indicating the organ in which follicular cell hyperplasia was observed. Also, F0 generation makes no sense as used in the >5-10 dose; F0 animals are the animals that are enrolled in the study; do you mean F1 generation?

RESPONSE: *This comment refers to Figure 1-1 and Figure 1-2 in section 1.2 (Summary of Health Effects). A sentence was added to section 1.2 which reads "Figure 1-1 and Figure 1-2 demonstrate that the minimal risk levels (MRLS) are established below any doses at which effects have been demonstrated." In order to define the term "MRL" which is presented in these figures. In addition, a footnote was added to the figure defining the acronym in the figure. In addition, the peer reviewer is correct and the text regarding the decrease in percent of males is specific to the F1 generation. The typo has been corrected.*

COMMENT: MF, BMCL10 and BMCL1SD should also be defined in footnote to this table.

RESPONSE: *The reviewer suggests these additions to the footnote for Table 1-1 in section 1.3 (Minimum Risk Levels (MRLs). MF, SD, and BMCL have been added to the footnotes.*

COMMENT: Should be defined initially in Chapter 1. It is fine to re-iterate these definitions here, and I encourage you to leave them in here.

RESPONSE: *This comment refers to the term intermediate in the following sentence in section 2.1 (Introduction): "These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days)."*

ATSDR's definition of acute (≤14 days), intermediate (15-364) and chronic (≥365) are now defined at first use in chapter one.

COMMENT: Change to "shown" or "demonstrated"

RESPONSE: *This comment refers to the use of observed in the following sentence from the renal bullet in section 2.1 (Introduction): "In addition, experimental animal studies have observed increases in kidney weight and degenerative changes in the cortical tubules." The text in question has been changed to now read "Several experimental animal studies have demonstrated increases in kidney weight and degenerative changes in the cortical tubules"*

COMMENT: Why would we expect to see sperm in urine?

RESPONSE: *This comment refers to the effects recorded in the Levin et al. 1988 entry in Table 2-2, Figure key 3. The text states "Sperm were not detected in the urine of treated rats between 32 and 48 days after treatment."*

This study continuously monitored sperm production by connecting rat vas deferns with their urinary bladder, allowing the researchers to evaluate sperm in urine. However, given the confusion with the text as written in the LSE table the entry was edited to now reads "Cessation of sperm output 32 days after exposure" to improve understandability of the effect observed by the researchers.

COMMENT: I am assuming that the first sentence refers to human studies……..

RESPONSE: *This comment refers to the first sentence in section 2.3 (Body Weight). The sentence states "No studies were located evaluating body weight effects of nitrobenzene exposure following inhalation, oral or dermal exposure."*

The reviewer is correct. The sentence now read "No human studies were located…" to improve clarity

COMMENT: Incomplete thought here. Not sensitive compared to male mice? Humans? Rats?

RESPONSE: *This comment refers to the statement "Smith et al. (1967) argued that female CD1 mice were not sensitive to effects of nitrobenzene on methemoglobinemia" in section 2.7 (Hematological).*

The sentence in question was revised and now reads "Smith et al. (1967) argued that nitrobenzene is a "poor methemoglobin-forming agent" in female CD1 mice."

COMMENT: Define?

RESPONSE: *The reviewer suggests defining torticollis, which is used in the following sentence in section 2.15 (Neurological): "With oral exposure around 100 ppm Sprague-Dawley rats experienced torticollis, circling movement and abnormal gait (Mitsumori et al. 1994)." A definition was added to the sentence in question and reads "a condition in which the neck muscles are contracted causing the head to tilt to one side)"*

COMMENT: Incomplete sentence as written. Remove "Though"?

RESPONSE: *This comment refers to the sentence "Though all animals were dead or moribund with treatment of 1.6 g/kg or greater by the end of the experiment" in section 2.15 (Neurological). The word "though" was removed as suggested.*

COMMENT: Please double check—Tania's last name is usually presented as Carreon-Valencia

RESPONSE: *This comment refers to the following sentence in section 2.18 (Cancer): "Carreon et al. (2014) assessed a cohort of workers occupationally exposed to o-toluidine, aniline and nitrobenzene at a rubber chemical manufacturing plant in New York." The PDF of this study lists her last name as Carreon. The name was changed from Carreon to Carreón throughout the profile.*

COMMENT: Should be 2.20, I believe

RESPONSE: *This comment refers to the cross-reference in the following sentence from section 2.18 (Cancer): "As described in the Genotoxicity section (see 2.19), the evidence is fairly conclusive that nitrobenzene does not cause point mutations, as the results of many Ames tests, with and without S9 activation (Anderson and Styles 1978; Assmann et al. 1997; Bonnefoy et al. 2012; Dellarco and Prival 1989; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Hughes et al. 1984; Vance and Levin 1984)." The reviewer is correct, and the section number has been updated to 2.20.*

COMMENT: reference missing

RESPONSE: *This comment refers to the following sentence in section 2.18 (Cancer): "For example, Li et al. (2003b) demonstrated that nitrobenzene can form adducts with hepatic DNA in mice and several studies demonstrated dose-dependent increases in chromosomal aberrations and increases in micronuclei." Citations were clarified and added to this sentence. Specifically, the sentence now reads "For example, Li, Wang, et al. (2003) demonstrated that nitrobenzene can form adducts with hepatic DNA in mice and several studies demonstrated dose-dependent increases in chromosomal aberrations and increases in micronuclei (Huan et al., 1995, 1996; Bonacker et al., 2004; Robbiano et al, 2004).*

COMMENT: reference missing

RESPONSE: *This comment refers to the following sentence in section 2.18 (Cancer): "However, unscheduled DNA synthesis was not observed in genotoxicity evaluations of nitrobenzene." Citations were added to this sentence and it now reads "DNA in mice and several studies demonstrated dose-*

dependent increases in chromosomal aberrations and increases in micronuclei (Huan et al., 1995, 1996; Bonacker et al., 2004; Robbiano et al, 2004)."

COMMENT: Incomplete sentence**.**

RESPONSE: *This comment refers to the following sentence in section 2.18 (Cancer): "Whereas, Ohkuma and Kawanishi (1999) found that nitrosobenzene, a metabolite of nitrobenzene, can cause DNA damage in the presence of NADH and Cu2+ by using an in vitro study with calf thymus DNA."*

The sentence was modified for clarity and now reads "Whereas, Ohkuma and Kawanishi (1999) found that nitrosobenzene, a metabolite of nitrobenzene, can cause DNA damage in the presence of NADH and Cu2+ in an in vitro study using calf thymus DNA"

COMMENT: The word **"**data" is plural.

RESPONSE: *This comment refers to the following sentence in section 2.20 (Genotoxicity), in the subsection about clastogenicity and aneugenicity: "While the evidence for a lack of mutagenicity of nitrobenzene, without co-mutagens, is strong, the data on clastogenicity is less so, with the majority of the evidence suggesting a potential genotoxic effect on the chromosome." The word "is" has been changed to "are" in response to this comment.*

COMMENT: I have no idea what this is and it's not in the abbreviation list

RESPONSE: *This comment refers to the use of CREST in the following sentence in the clastogenicity and aneugenicity section of section 2.20 (Genotoxicity): "CREST analysis suggested the micronucleus effects were aneugenic, not, not clastogenic."*

In response to this comment ATSDR spelled out the CREST acronym and added a brief definition in Section 2.20 (Genotoxicity). Specifically, the sentence now reads "Minimal-effect-concentrations of nitrobenzene appeared to be as low as 0.01 µM and no-effect-concentrations were between 0.001 and 0.005 µM. CREST (Calcinosis, Raynaud's phenomenon, Esophageal dysfunction, Sclerodactyly, and Telangiectasias; a staining method to evaluate between aneugens and clastogens) analysis suggested the micronucleus effects were aneugenic, not, clastogenic." ATSDR also added the definition of this term to Appendix F.

COMMENT: In lymphocytes? In a particular tissue? Important to indicate where……..

RESPONSE: *This comment refers to the following sentence in the clastogenicity and aneugenicity section of section 2.20 (Genotoxicity), questioning where in rats this effect takes place: "Additionally, Robbiano et al (2004) observed a dose-dependent increase in micronucleated cells in rats due to broken and detached chromosomes separated from the spindle apparatus in rats treated with 300 mg/kg nitrobenzene via gavage."*

These findings were from a study evaluating rat kidney cells. This has been specified in the Profile and the sentence in question now reads "Additionally, Robbiano et al (2004) observed a dose-dependent increase in micronucleated kidney cells in rats due to broken and detached chromosomes separated from the spindle apparatus in rats treated with 300 mg/kg nitrobenzene via gavage."

COMMENT: Please don't refer to doses in an in vitro study. "…..in both human and rat kidney cells incubated with concentrations of 0.125……"

RESPONSE: *This comment refers to the following sentence in the DNA Damage section of section 2.20 (Genotoxicity): "This observation was made in both human and rat kidney cells with doses of 0.125 to 0.50 mM nitrobenzene." In response to this comment, we edited the sentence as suggested by the peer reviewer and it now reads "This observation was made in both human and rat kidney cells incubated with concentrations of 0.125 to 0.50 mM nitrobenzene."*

COMMENT: Is 'metabolism' the correct word here? Seems that "signaling" would be more appropriate.

RESPONSE: *This comment refers to the following sentence in the DNA Damage section of section 2.20 (Genotoxicity): "Several investigators have suggested that nitrobenzene may act as a promotor (via intracellular metabolism), since the reactive intermediates generated during its metabolism may have potential to initiate, promote and/ or accelerate the progression of non-neoplastic or neoplastic changes in cells (Dreher and Junod, 1996; Feig et al 1994; Guyton and Kensler, 1993; Kensler et al 1989)." Upon closer review of this paragraph ATSDR determined it should be deleted. The citations quoted are either not primary articles or do not specifically mention nitrobenzene.*

COMMENT: "Initiate" seems wrong here. Promoters can promote and/or accelerate, but they have nothing to do with initiation, by definition.

RESPONSE: *This comment refers to the following sentence in the DNA Damage section of section 2.20 (Genotoxicity): "Several investigators have suggested that nitrobenzene may act as a promotor (via intracellular metabolism), since the reactive intermediates generated during its metabolism may have potential to initiate, promote and/ or accelerate the progression of non-neoplastic or neoplastic changes in cells (Dreher and Junod, 1996; Feig et al 1994; Guyton and Kensler, 1993; Kensler et al 1989)." Upon closer review of this paragraph ATSDR determined it should be deleted. The citations quoted are either not primary articles or do not specifically mention nitrobenzene.*

COMMENT: I wish that all abbreviations were defined in the text, as has been done here.

RESPONSE: *This comment refers to the following sentence in section 3.1.1 (Absorption): "Patel et al. (2008) reported a case of extremely high levels of methemoglobin (MetHb) (66.7%) in a 20-year-old male 16 h after ingesting about 75 mL nitrobenzene." In response to this comment, the profile was reviewed to define abbreviations at first use in each chapter.*

COMMENT: Run on sentence

RESPONSE: *This comment refers to the following sentence in the second paragraph in section 3.1.1 (Absorption): "The authors speculated that due to massive ingestion of nitrobenzene, metabolism of the parent compound and its active metabolites was saturated which may have led to prolonged exposure to the active metabolite (Perera et al. 2009)." In response to this comment the sentence was split into two and now reads "The authors speculated that due to massive ingestion of nitrobenzene, metabolism of the parent compound and its active metabolites was saturated. This may have led to prolonged exposure to the active metabolite (Perera et al. 2009)."*

COMMENT: Urine, feces, both?

RESPONSE: *This comment refers to the following sentence in section 3.1.1 (Absorption): "A significant absorption of nitrobenzene from the gastrointestinal tract appeared in Fischer-344 and CD rats with the recovery of 72- 88% in 72 h, and lesser in B6C3F1 mice with 54%." The sentence was edited to clarify this was in urine.*

COMMENT: I don't see the need for this abbreviation—not used again.

RESPONSE: *The reviewer suggests removing the acronym "BBMV" from the following sentence in section 3.1.1 (Absorption): "An in vitro study investigated the mechanisms of nitrobenzene absorption using brush border membrane vesicles (BBMVs) isolated from the small intestines of Sprague-Dawley rats (Alcorn et al. 1991)." The text "(BBMV)" was deleted.*

COMMENT: This seems HUGE—please confirm that mg is correct.

RESPONSE: *This comment refers to the dose 10-19 mg in the following sentence in section 3.1.1 (Absorption): "For instance, naked subjects exposed to a chamber concentration of 10 µg/L nitrobenzene had an estimated absorbed dose from 10– 19 mg, while wearing normal working clothes reduced absorption of nitrobenzene by 20–30%." The paper was consulted, and it was confirmed that mg is the correct unit presented in the paper.*

COMMENT: ????

RESPONSE: *This comment refers to the use of DFG in the following sentence in section 3.1.1 (Absorption): "DFG (2012) estimated the absorption of 25 mg per day by persons exposed to an air concentration of 1 ml/m3 nitrobenzene at work through skin (one third) and inhalation (two thirds) (DFG 2012)." DFG stands for Deutsche Forschungsgemeinschaft and is a Germany research company. The acronym has been defined in the text in question.*

COMMENT: Again, I suspect that this should be microgram values, not mg values.

RESPONSE: *This comment refers to the concentrations in the following statement in section 3.1.2 (Distribution): "Nitrobenzene was found in stomach, liver, brain and blood with the highest concentration in liver (124 mg/kg tissue), and brain (164 mg /kg tissue) in autopsies of 5 patients which had died from nitrobenzene poisoning (Wirtschafter and Wolpaw 1944)." Review of the citation resulted in a correction being made to the data presented. The concentration in the liver is now presented as 0.124 mg/kg and the brain is 0.164 mg/kg tissue.*

COMMENT: And was followed by phase II reactions?

RESPONSE: *This comment refers to the following statement in the first paragraph of section 3.1.3(Metabolism): "Reduction of the nitro group yields nitrosobenzene, phenylhydroxylamine, and aniline, and followed phase II reactions involving the replacement of a nitro group by glutathione, and the formation of sulfated or glucuronidated conjugates." The word "by" was added to the sentence as suggested by the reviewer.*

COMMENT: Very poor quality image. Two of the structures are wrong. In the upper left, the OSO3H group should have carbon bonded to sulfur. Phenylhydroxylamine (on the right, near the middle of the diagram, the Nitrogen should be bonded t the carbon, not a hydrogen.

RESPONSE: *This comment refers to Figure 3-3 in section 3.1.3 (Metabolism). The functional groups are presented accurately in condensed formula format, where the first atom listed is bonded to the carbon. We believe the commenter is incorrectly assuming that the functional groups are being presented as skeletal formula. While no revisions have been made to this figure, we have updated the image to have better quality. Other images in Chapter 3 have been updated to have improved quality and are 508 clearance compliant.*

COMMENT: This figure would be improved if nitrobenzene was added to the figure.

RESPONSE: *This comment refers to Figure 3-6 in section 3.1.3 (Metabolism). Nitrobenzene was depicted in the original figure though it was not labeled. The figure has been updated to match the data from Holder 1999 (the original source of the data) with nitrobenzene labeled.*

COMMENT: Toxicologists frown on the use of the word "subacute". Think about it. If acute is a single, potentially large exposure, what could subacute possibly mean. The more acceptable term is short-term repeated dose (study/exposure).

RESPONSE: *This comment refers to the use of the term "subacute" in the following sentence in section 3.1.6 (Animal-to-Human Extrapolations): "Two major metabolites, p-aminophenol and p-nitrophenol, have also been shown persistent in urine of patients after acute and subacute poisoning. Only 20 or 30% of nitrobenzene dose was excreted as its metabolites from humans in the urine (Piotrowski 1977)."*

Given the sentence in question is referring to poisoning we removed the acute and subacute terminology.

COMMENT: ????

RESPONSE: *This comment refers to use of "peroral" in the following sentence in section 3.4 (Interactions with Other Chemicals): "Smyth et al. (1969) demonstrated synergism between orally administered nitrobenzene and six other common industrial compounds in rat studies using death (peroral LD50) as the endpoint." The term peroral means "through the mouth" and was indicated the LD50 derived was one for oral exposure. The term peroral has been changed to oral in the sentence in question to improve the readability of the document.*

COMMENT: ?????

RESPONSE: *The reviewer questions the following sentence, particularly "capacity," in section 3.4 (Interactions with Other Chemicals): "The ethanol extract of Euphobia hirta is a suggested antioxidant against nitrobenzene-induced nephrotoxicity, which ameliorates renal damage, capacity (Suganya et al. 2011)." The word "capacity was a typo and has been removed.*

COMMENT: Highly soluble in water? Slightly soluble in water? Looking at the structure, I would not expect high water solubility. Clarification would be appreciated.

RESPONSE: *The reviewer requests clarification on the following sentence in section 4.2 (Physical and Chemical Properties): "It is soluble in water and most other organic solvents and it represents a fire hazard." In section 5.3.2 (Water), the text states that nitrobenzene is sparingly soluble in water. This sentence was updated to say "It is sparingly soluble in water…"*

COMMENT: Water is not an organic solvent. Remove "other".

RESPONSE: *The reviewer suggests removing "other" from the following sentence in section 4.2 (Physical and Chemical Properties): "It is soluble in water and most other organic solvents and it represents a fire hazard." "Other" was removed from this sentence.*

COMMENT: Suggest mentioning that NPL sites are also known as Superfund sites.

RESPONSE: *The reviewer suggests this clarification in the first paragraph in section 5.1 (Overview). This text is boilerplate, which is dictated by ATSDR's Guidance on Developing Toxicological Profiles, and therefore edits were not made to this paragraph.*

COMMENT: Where is the reader pointed to this table in the text?

RESPONSE: *This comment refers to table 5-1 in section 5.2.1 (Production). A sentence describing the table was inserted before the table in section 5.2.1 that says, "Table 5-1 lists the facilities in each state that manufacture or process nitrobenzene, the intended use, and the range of maximum amounts of nitrobenzene that are stored on site."*

COMMENT: Chapter 7 really doesn't talk about disposal—it's a very brief description of a number of regulations.

RESPONSE: *This comment refers to the following sentence in section 5.2.4 (Disposal): "Because nitrobenzene is listed as a hazardous substance, disposal of waste nitrobenzene is controlled by a number of federal regulations (see CHAPTER 7)." To address this comment, more information was added to chapter 7 on the disposal regulations that apply to nitrobenzene. "The Resource Conservation and Recovery Act (RCRA) identifies nitrobenzene as a toxic waste with toxicity and a hazardous constituent of waste. Because nitrobenzene is listed as a hazardous substance, the storage, transportation, treatment and disposal of waste nitrobenzene is controlled by EPA. It has been assigned the hazardous waste codes of U169, F004, K083, K103, K104 (NTP 2016). Since nitrobenzene is assigned the hazardous waste code F004, nitrobenzene wastes are prohibited from underground injection unless the waste contains less than 1 percent of nitrobenzene (EPA 2020). Nitrobenzene is also subject to land disposal restrictions (EPA 2020)."*

COMMENT: Why are we talking about 1,2-dichloroethane here? Are the numbers in the table relevant to 1,2-dichloroethane, or to nitrobenzene? Where in the text is the reader pointed to this table?

RESPONSE: *This comment refers to the title of Table 5-2 in section 5.3 (Releases to the Environment), which reads "Releases to the Environment from Facilities that Produce, Process, or Use 1,2-Dichloroethane[a]." This table refers to nitrobenzene data and the reference to 1,2-dichloroethane was a mistake that wasn't updated when we copied over the table formatting. The table was checked against the nitrobenzene TRI data and it is correct. The table was also moved so that it appears after being mentioned in the text. The table is now located in section 5.3.1 (Air).*

COMMENT: Correct as written??

RESPONSE: *This comment refers to the footnote "Source: TRI17 2019; Data are from 2017" on Table 5-2 in section 5.3 (Releases to the Environment). This is correct. In the downloaded data (which is a spreadsheet in the Endnote library called TRI Facility Data), EPA suggests citing this data as "United States Environmental Protection Agency. (2019). TRI Explorer (2017 Updated Dataset (released April 2019) [Internet database]. Retrieved from https://www.epa.gov/triexplorer, (June 25, 2019)." The in-text citation and the reference were updated to follow ATSDR's citation format.*

COMMENT: TRI has been used dozens of times previously and is finally defined here. Please define abbreviations the first time that they are used in each chapter. Not everyone is going to read this entire document and it's really inconvenient to have to go repeatedly to a glossary.

RESPONSE: *This comment refers to the use of TRI in the following sentence in section 5.3 (Releases to the Environment): "The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005)." The TRI has been defined prior to its first use in every instance where it is used.*

COMMENT: Ah-ha—this addresses one of my previous questions about nitrobenzene's solubility in water.

RESPONSE: *This comment refers to the following sentence in section 5.3.2 (Water): "Although nitrobenzene is sparingly soluble in water [1,900 ppm at 20°C (Verschueren 1985); 2,090 ppm at 25°C (Banerjee et al. 1980)], its pungent, characteristic odor ["bitter almonds," (Windholz et al. 1983); "shoe polish," (Ruth 1986)] is detectable at water concentrations as low as 30 ppb (EPA 1980a)." The commenter is referring to their comment in chapter 4 concerning the solubility in water, and whether nitrobenzene is highly soluble or slightly soluble. The language in this instance and in chapter 4 has been updated to state that nitrobenzene is sparingly soluble for consistency.*

COMMENT: Are the zeros all correct in this paragraph? I don't understand why some paragraphs are bolded and some are not (that has puzzled me throughout the document. Also, Table 5-2 was presented many pages ago and relates to 1,2-dichloroethane.

RESPONSE: *This comment refers to the boilerplate language in section 5.3.2 (Water), which states "Estimated releases of 0 pounds (0 metric tons) of nitrobenzene to surface water from 18 domestic manufacturing and processing facilities in 2017, accounted for about 0% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2019). An additional 0 pounds (0 metric tons) were released to publicly owned treatment works (POTWs) (TRI17 2019). These releases are summarized in Table 5 2." Some paragraphs are bolded to indicate boilerplate text; the formatting will be removed for the public draft. The title of Table 5-2 has been updated to reflect the correct chemical (see previous comment about this issue). The zeros are correct. The facilities that reported to TRI reported 0 pounds of releases to surface waters. The facilities did not report releases to POTWs, so the language has been changed to reflect this. The profile now states "Estimated releases of 0 pounds (0 metric tons) of nitrobenzene to surface water from 18 domestic manufacturing and processing facilities in 2017, accounted for about 0% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2019). The facilities did not report releases to publicly owned treatment works (POTWs) (TRI17 2019). These releases are summarized in Table 5 2."*

COMMENT: I have no idea what this means and I doubt that most other readers would either.

RESPONSE: *This comment refers to the use of "Glycine max L Merr" in the other media paragraphs in section 5.4.1 (Transport and Partitioning). The sentence says, "The relatively rapid uptake of 14C-labeled nitrobenzene into mature soybean (Glycine max L Merr) plants was reported by McFarlane et al. (1987; 1987) and Nolt (1988)." Glycine max L Merr is the scientific name for soybeans. The text was updated to italics to make it more clear that it is the species name.*

COMMENT: Table should be formatted to fit on one page. This applies to all tables in the document.

RESPONSE: *This comment refers to Table 5-3 in section 5.5 (Levels in the Environment). Formatting changes were made so that this table fits on one page. All other tables that could be formatted to fit on one page were also edited.*

COMMENT: Please define

RESPONSE: *The reviewer suggests defining "CERCLA" as used in the following sentence in chapter 6 (Adequacy of the Database): "Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of nitrobenzene is available." The definition of CERCLA is now included in the text where it reads "(the Comprehensive Environmental Response, Compensation and Liability Act)"*

COMMENT: The repetition in this document is getting annoying. We saw this in Chapters 1 and 2—does it need to be repeated here?

RESPONSE: *This comment refers to Figure 6-1 in section 6.1 (Existing Information on Health Effects). The format of the profile follows ATSDR's Guidance on the Development of Toxicological Profiles and therefore no changes have been made in response to this comment.*

COMMENT: Remove period

RESPONSE: *The reviewer suggests removing the period after Figure 6-1 in the following sentence in section 6.2 (Identification of Data Needs): "Missing information in Figure 6 1. should not be interpreted as a "data need.' The period has been removed.*

COMMENT: Not a toxicological term. Significant???

RESPONSE: *This comment refers to the use of "stark" in the following sentence in the respiratory health effects paragraph in section 6.2 (Identification of Data Needs): "For example, there was a stark increase in the bronchiolization of the alveoli in mice expose to nitrobenzene and increase pigmentation and degeneration of the olfactory epithelium." The word "stark" has been replaced with the word "significant."*

COMMENT: These are not endocrine effects, per se. They are evidence of exposure and possibly toxicity. One can only claim endocrine effects if there are hormonal changes documented.

RESPONSE: *This comment refers to the following sentence in the endocrine health effects paragraph in section 6.2 (Identification of Data Needs): "Additionally, chronic nitrobenzene inhalation resulted in thyroid follicular cell hyperplasia in Cattley et al. (1994)." According to ATSDR's Guidance Document for the Development of Toxicological Profiles, thyroid hyperplasia is considered an endocrine effect. However, to address the reviewers concern the following text was added to the sentence in question and now reads "Additionally, chronic nitrobenzene inhalation resulted in thyroid follicular cell hyperplasia in Cattley et al. (1994) which may indicate toxicity to the thyroid"*

COMMENT: Insert reference—Carreon et al.

RESPONSE: *The reviewer suggests inserting this reference for the following statement in the cancer health effects paragraph in section 6.2 (Identification of Data Needs): "In the current profile only one epidemiological study was located, which also included exposure to two other chemicals (aniline and o-toluidine)." The reference to Carreon et al. 2014 was added to this sentence.*

COMMENT: I disagree completely with this statement. 2 rodent studies are described, and they are old studies. NHANES data suggest possible exposure, but are not definitive, given other potential sources of metabolites.

RESPONSE: *This comment refers to the following statement in the Children's Susceptibility paragraph in section 6.2 (Identification of Data Needs): "Data needs related to both prenatal and childhood exposures, and developmental effects expressed whether prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above." This text is standardized boilerplate text as dictated by the Guidance for the Preparation of Toxicological Profiles. The text is referring to the data needs section of the developmental effects where it is stated that it would be beneficial to further study potential developmental effects. Given the lack of data on this topic and the text in question is boilerplate text no revisions were made in response to this comment.*

COMMENT: Please define

RESPONSE: *The reviewer suggests defining MRLs as used in the first sentence in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure." A definition was now added the text reads "MRLs (Minimal Risk Levels)…"*

COMMENT: Per the tables in this worksheet, BMDs are also used to derive MRLs.

RESPONSE: *This comment refers to the following sentence in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach" The reviewer is correct. The sentence has been modified to read "MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach or benchmark dose modeling with applied uncertainty factors"*

COMMENT: Define. Most readers would have no idea what this means.

RESPONSE: *The reviewer suggests defining "ecchymosis" as used in the following sentence in the acute inhalation MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "However, the incidence of litters with one or more fetuses with external variations was elevated at 40.0 ppm for ecchymosis on the trunk (but not on the head or extremities)." The term was defined as (discoloration of the skin due to bleeding underneath).*

COMMENT: Route is not needed in column header as it's indicated in the title of the table

RESPONSE: *This comment refers to the table header "Duration/route" in Table A-1 in Appendix A (ATSDR Minimal Risk Levels and Worksheets). The suggested edit was made, and the text was removed.*

COMMENT: NO EFFECT IS INDICATED

RESPONSE: *This comment refers to the Effect column for the New Zealand white rabbits hematological effects entry, citing Biodynamics 1984, in Table A-1 in Appendix A (ATSDR Minimal Risk Levels and Worksheets). There is no effect indicated given that the data presented in the table are for a NOAEL for hematological effects. No revisions were made in response to this comment.*

COMMENT: Route not needed in header of column as it's indicated in the table's title

RESPONSE: *This comment refers to the table header "Duration/route" in Table A-4 in Appendix A (ATSDR Minimal Risk Levels and Worksheets). The suggested edit was made, and the text was removed.*

COMMENT: ?????

RESPONSE: *This comment refers to the "M: M:" in Table A-4 in Appendix A (ATSDR Minimal Risk Levels and Worksheets) corresponding to the effect column for the hematological effects in F344 Rats, citing Hamm Jr. 1984. The "M: M:" was a typo with an extra M which was deleted. Additionally, a footnote was added to the table indicating "M = males and F= females."*

COMMENT: Subjective. Remove "only".

RESPONSE: *The reviewer suggests removing "only" from the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Levels and Worksheets): "Further, with only 5 ppm exposure to nitrobenzene 58/67 male mice and 55/60 female mice displayed bronchiolization of the*

alveoli and at 50 ppm, the highest dose in the study, 62/66 male and 62/62 female mice displayed this effect." The suggested edit was made, and the word "only" was removed from the sentence.

COMMENT: Subjective. Remove "just"

RESPONSE: *The reviewer suggests removing "just" from the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Levels and Worksheets): "). There was also a noted increase in extramedullary hematopoiesis with just 1 ppm exposure to nitrobenzene, though there was a high incidence of this outcome in controls." The suggested edit was made, and the word "just" was removed from the sentence.*

COMMENT: Subjective. Remove "only"

RESPONSE: *The reviewer suggests removing "only" from the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Levels and Worksheets): "The same effects were seen only at the 50 ppm exposure level in female mice." The suggested edit was made, and the word "only" was removed from the sentence.*

COMMENT: Define, or use a term that the ordinary reader would understand

RESPONSE: *The reviewer suggests defining "spongiosis hepatis" as used in the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Levels and Worksheets): "The occurrence of spongiosis hepatis was also increased in 25-ppm nitrobenzene-exposed CD rats." A definition was added to the sentence which now reads "The occurrence of spongiosis hepatis (cystic degeneration of liver cells) was also increased in 25-ppm nitrobenzene-exposed CD rats"*

COMMENT: Previous page mentions Sprague Dawley and F344 rats. What are CD rats?

RESPONSE: *This comment refers to the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Levels and Worksheets): "The incidence of rats with Kupffer cell pigmentation was increased at all nitrobenzene exposure concentrations, with the lowest dose being 1 ppm in CD rats (Cattley et al. 1994)." In Cattley et al. (1994) they refer to the Sprague-Dawley rats as CD rats. To negate confusion throughout the profile we have changed CD rats to Sprague-Dawley rats. Included the following in the text "Cattley et al. (1994) refers to Sprague-Dawley rats as CD rats, but this report calls them Sprague-Dawley rats for consistency."*

COMMENT: CD rats????? Same comment throughout this table

RESPONSE: *This comment refers to the CD Rat respiratory effects entry in Table A-6 in Appendix A (ATSDR Minimal Levels and Worksheets). In Cattley et al. (1994) they refer to the Sprague-Dawley rats as CD rats. To negate confusion throughout the profile we have changed CD rats to Sprague-Dawley rats. Included the following in the text "Cattley et al. (1994) refers to Sprague-Dawley rats as CD rats, but this report calls them Sprague-Dawley rats for consistency."*

COMMENT: I don't understand the values in the NOAEL and LOAEL columns. What does "M" mean?

RESPONSE: *This comment refers to the notation in Table A-6 in Appendix A (ATSDR Minimal Levels and Worksheets). The reviewer points to the NOAEL entry for renal effects in CD Rats as an example, which is 24.8M (4.4). To clarify a footnote was added to Table A-6 which reads "M = Male"*

COMMENT: Not a proper toxicology term Significant?

RESPONSE: *This comment refers to the use of "stark" in the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "Given the stark increase in respiratory effects, olfactory degeneration and alveolar bronchiolization were evaluated as critical effects." The word "stark" has been replaced with the word "significant."*

COMMENT: Same comment as above

RESPONSE: *This comment refers to the use of "stark" in the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "From the effects seen in Cattley et al. (1994) it is clear a number of effects occur around 5 ppm of exposure including stark increases in the incidence of bronchiolization of the alveolar wall in male and female mice along with olfactory degeneration, pigment deposition in male Sprague-Dawley rats, centrilobular hepatocytomegaly and eosinophilic foci in the livers of male F344 rats." The word "stark" has been replaced with the word "significant."*

COMMENT: No first person in this document, please

RESPONSE: *The reviewer suggests editing the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets) to remove first person pronouns: "Further, extramedullary hematopoiesis had a very high background rate in controls (77%) and we therefore did not select it as the critical effect."*

COMMENT: Same comment as at top of page

RESPONSE: *This comment refers to the use of "stark" in the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "Given the stark increase in bronchiolization of the alveolar wall and the increased degeneration of the olfactory epithelium the respiratory effects were evaluated for potential to inform the MRL for nitrobenzene." The word "stark" has been replaced with the word "significant."*

COMMENT: Remove 1st person

RESPONSE: *The reviewer suggests editing the following sentence in the chronic inhalation MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets) to remove first person pronouns: "Although there are 4 parameters in this model, one is bounded, allowing us to use this model for derivation of the MRL." The sentence has been edited to read "Further, extramedullary hematopoiesis had a very high background rate in controls (77%) and therefore it was not selected as the critical effect."*

COMMENT: Define. Even if you take my advice and define it earlier in the document, the reader may not read the whole document.

RESPONSE: *The reviewer suggests defining "intermediate" as used in the following sentence in the intermediate oral MRL Worksheet in Appendix A (ATSDR Minimal Risk Levels and Worksheets): "An intermediate -duration oral MRL of 0.02 mg/kg/day was derived for nitrobenzene based on evidence of increased methemoglobin levels in male F344 rats administered nitrobenzene via gavage for 90 days (NTP 1983a)." The text "(15-365 days)" was added after intermediate-duration to the sentence in question.*

COMMENT: Did not note any GW dosing in this table

RESPONSE: *This comment refers to the definition of GW (gavage in water vehicle) in the footnote for Table A-9 in Appendix A (ATSDR Minimal Risk Levels and Worksheets). The footnote reading GW = gavage in water vehicle has been removed from Table A-9.*