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 post-comment draft of the Toxicological Profile for Nitrobenzene (addressing the MRL revision only) were:

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Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

Chronic Inhalation MRL

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain. 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. 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 1: I agree with the proposed chronic inhalation MRL. The study upon which it is based is well conducted and the best available source of chronic toxicity data. The dose response data evaluation is consistent with current EPA guidelines and the calculations to derive the Point of Departure and application of uncertainty factors appear to be correct. I agree with each component of the total uncertainty factor. The use of a LOAEL as the Point of Departure and potential variability in human sensitivity each merit a factor of 10, and a factor of 3 for animal to human extrapolation is consistent with EPA guidelines when dosimetric adjustment has been made.

RESPONSE: *No response needed.*

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

COMMENT 2: There are no aspects of the database adjustment that need to be addressed.

RESPONSE: *No response needed.*

Unpublished Study

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

COMMENT 3: The study used an adequate number of animals. Details regarding animal care are not included in the report but there is nothing in the report that is concerning.

RESPONSE: *No response needed.*

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

COMMENT 4: The possibility of other causes for observed adverse health effects, including death, were accounted for by using appropriate control groups. Also, the possibility that some of the observed pathology was not treatment related was considered and discussed, for example in Appendix W.

RESPONSE: *No response needed.*

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

COMMENT 5: The number of dose groups (controls plus 3) is typical for a chronic bioassay. I consider this sufficient. The dose range examined was able to identify the principal adverse effects of nitrobenzene and is sufficient for hazard identification. The dose range did not include a NOAEL for the most sensitive non-cancer effects. I do not consider this a serious limitation of the study as an MRL can be [and was] derived through incorporation of an appropriate uncertainty factor.

RESPONSE: *No response needed.*

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

COMMENT 6*:* The study was adequately designed and reported.

RESPONSE: *No response needed.*

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

COMMENT 7*:* The report summary consists primarily of a brief description of the experimental approach and an overview of the findings. Any conclusions are in the form of statements regarding the adverse effects observed and these appear to be accurate based upon information presented in the body of the report. There are no statements that I disagree with.

RESPONSE: *No response needed.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses and Reviewer Comments

Chronic Inhalation MRL

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain. 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. 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 1: Agree with the newly derived chronic inhalation MRL. Agree with each of the components of the total uncertainty factor for the chronic inhalation MRL and other MRLs.

RESPONSE: *No response needed.*

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

COMMENT 2: Please note that there is lack of clarity regarding the critical effect. In Table 1-1 and in some parts of the Appendix A section for Chronic Inhalation MRL, the finding is variably referred to as "Hyperplasia of the nasal olfactory epithelium" when it appears that the finding is epithelial hyperplasia in Level 1 nasal sections from which it inferred as "squamous" epithelial hyperplasia (see text pg 48 line 15 and Appendix table A-9; the designation "squamous" is a reasonable inference based on location in Level 1, although not explicity stated as "squamous" in the report). See report screen shots (attached). I believe that Level 1 sections do not contain olfactory epithelium. This lack of clarity should be addressed in subsequent revisions.

Also, you may wish to add citation to the erratum reported by Cattley et al (1994) [see Fundam Appl Toxicol 1995 Apr;25(1):159], although the correction did not affect any of the data used in the Profile or the newly derived chronic inhalation MRL.

RESPONSE: *Table 1-1 and Appendix A (Critical Effect and MRL Summary) were corrected to show "hyperplasia of the nasal squamous epithelium" when referring to the critical effect in male CD rats for the chronic inhalation MRL. The unpublished report (CIIT 1993) reported the lesion in question as epithelial hyperplasia of the mucosa (Table S3b and results section), and the publication (Cattley et al. 1994) reported the lesion as squamous epithelial hyperplasia (bottom of first column on page 334). This error was not identified elsewhere in the profile.*

The erratum citation was added throughout the profile.

> ***MRL Summary:*** A chronic-duration inhalation MRL of 0.0002 ppm was derived for nitrobenzene based on hyperplasia of the squamous epithelium in male CD rats following exposure to nitrobenzene via inhalation for 2 years (Cattley et al. 1994, 1995; CIIT 1993).

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

COMMENT 3: The study used an adequate number of animals, including 50 animals/group/sex for terminal sacrifice and 10 animals/group/sex for interim sacrifice. In the case of the mouse study, there was significant early mortality, unrelated to exposure level, which was attributed to animal husbandry procedures. This was rectified by cage modifications. As a result, the interim sacrifice in the mouse study was canceled to ensure adequate numbers for the terminal sacrifice. The number of animals surviving for most or all of the study duration were sufficient to demonstrate non-cancer and cancer endpoints.

RESPONSE: *No response needed.*

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

COMMENT 4: The report contains detailed accounting of all animals including cause of death in Appendix Tables 3B1, 3B2, and 3B3.

RESPONSE: *No response needed.*

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

COMMENT 5: The study used a sufficient number of dose groups, 3 different exposure level groups plus one control group. The exposure levels were of sufficient magnitude to demonstrate noncancer and cancer endpoints with sufficient survival to the terminal sacrifice.

RESPONSE: *No response needed.*

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

COMMENT 6*:* The results of an initial independent audit are reported in Appendix V, which states that the studies were not compliant with GLP, due to issues in record keeping and error corrections, SOPs, and the role of the QAU. Based upon the recommendations of the independent audit, the CIIT study team addressed these issues and documented remedial actions. As a result of these remedial actions, in the final audit report, the independent audit team concluded that the results and methods presented in the final report are consistent with the study records and raw data, and the study records allow reconstruction of the conduct of the study.

Taken together, these inadequacies do not negate the utility of the studies as presented in the final report.

RESPONSE: *No response needed.*

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

COMMENT 7*:* Agreed with conclusions presented in the final report.

RESPONSE: *No response needed.*

Specific Comments on Toxicological Profile

COMMENT 8: Referring to the critical effect for the chronic inhalation MRL in Table 1-1, the Reviewer commented "Shouldn't this be "Squamous epithelial hyperplasia" to match the Appendix A."

RESPONSE: *Table 1-1 was corrected to show "hyperplasia of the nasal squamous epithelium" as the critical effect in male CD rats for the chronic inhalation MRL.*

COMMENT 9: Referring to the statement in Section 2.4—Nasal lesions occurred at all exposure concentrations (≥1 ppm) in rats included olfactory epithelial pigment deposition (F344 and CD) and squamous epithelial hyperplasia (CD rats)—the Reviewer commented "Inferred as "squamous" based upon location in Level 1 nasal sections."

RESPONSE: *While the unpublished report (CIIT 1993) reported the lesion in question as epithelial hyperplasia of the mucosa (Table S3b and results section), the publication (Cattley et al. 1994) reported the lesion as squamous epithelial hyperplasia (bottom of first column on page 334). No change needed.*

COMMENT 10: Referring to the statement in Section 2.7—Many of the effects induced by nitrobenzene result from production of methemoglobin, with the destruction of erythrocytes and oxidative stress that ensue—the Reviewer commented "Methemoglobinemia is a functional anemia related to oxygen retention. I don't think that destruction of erythrocytes "ensues" from methemoglobin production. What is the evidence that the methemoglobin causes erythrocyte destruction rather than the same nitrobenzene metabolites that oxidize Hbg-Fe can also cause oxidative damage to erythrocyte membranes?"

RESPONSE: *The text in Section 2.7 was revised for clarity as follows:*

> Many of the effects induced by nitrobenzene result from production of methemoglobin, along with the destruction of erythrocytes and oxidative stress.

COMMENT 11: Referring to the statement in Section 2.7—As the spleen is the primary site where damaged erythrocytes are scavenged, splenic congestion and hemosiderosis are common findings, and may result in depletion of lymphoid cells—the Reviewer commented "The spleen is an organ that can accommodate a marked increase in size. I think that lymphoid depletion may be a relative change, not an actual change."

RESPONSE: *The statement in Section 2.7 was revised for clarity to indicate that the lymphoid depletion may be relative.*

> As the spleen is the primary site where damaged erythrocytes are scavenged, splenic congestion and hemosiderosis are common findings, and may result in relative depletion of lymphoid cells**.**

COMMENT 12: Referring to the statement in Section 2.7—In mice, lymphoid depletion of the spleen was noted at ≥150 mg/kg/day—the Reviewer commented "Could this be relative rather than absolute?

Was spleen weight increased? Delete based upon above statement that "Because only one male rat survived in the 150 mg/kg/day dose group, results from this group are not informative."

RESPONSE: *In the study by NTP (1983a), spleen weight was not measured, so it is uncertain whether the effect was relative rather than absolute. The statement "Because only one male rat survived in the 150 mg/kg/day dose group, results from this group are not informative" refers to rats, not mice, so it is not relevant to the comment about lymphoid depletion in mice. No change was made.*

COMMENT 13: Referring to the statement in Section 2.7—Spleen congestion was seen in all treated rats but only in mice exposed to the highest dose (0.8 g/kg)—the Reviewer commented "Does this mean that it was seen in all 'groups of' treated rats?"

RESPONSE: *The text in Section 2.7 was revised as suggested.*

> Spleen congestion was seen in all groups of treated rats but only in mice exposed to the highest dose (0.8 g/kg).

COMMENT 14: Referring to the discussion of renal effects in Oladele et al. (2021) in Section 2.10, the Reviewer commented "I could not access the Oladele reference. Not sure of methods of histopathology- were all animals examined and reported by a qualified pathologist?"

RESPONSE: *Oladele et al. (2021) did not indicate whether all animals were examined for histopathology, nor did they describe the qualifications of the pathologist. No change was made.*

COMMENT 15: Referring to the discussion of mechanisms in Oladele et al. (2021) in Section 2.10, the Reviewer commented "Could not access this paper- it is not clear whether oxidative stress was established in this study or if these are secondary effects."

RESPONSE: *No change suggested or implied by the comment. No response needed.*

COMMENT 16: Referring to the statement in Section 2.13—Male F344 rats showed increases in the incidence of focal pancreatic acinar cell hyperplasia and diffuse hyperplasia of the parathyroid gland after exposure to 24.8 ppm nitrobenzene (Cattley et al. 1994; CIIT 1993)—the Reviewer commented "This lesion occurs in the exocrine pancreas and would be more appropriately placed in section 2.6 Gastrointestinal."

RESPONSE: *The statement regarding pancreatic acinar cell hyperplasia was removed from Section 2.13 and from the Endocrine subsection of Section 2.1 and added to Section 2.6, Gastrointestinal. This effect was also removed from the Endocrine LOAEL and added as a Gastrointestinal LOAEL in the Table 2-1 entry for F344 rats in the study by Cattley et al. 1994 (1995, CIIT 1993).*

> Section 2.1: **Endocrine:** Nitrobenzene effects on endocrine organs of laboratory rodents included increased incidences of adrenal cortical cell vacuolization and/or fatty changes in mice; increased basophilia of the adrenal medullary cells in rats; thyroid follicular cell hypertrophy, hyperplasia, and/or decreased serum thyroid-stimulating hormone (TSH) in rats and mice; and diffuse hyperplasia of the parathyroid gland in rats.

Section 2.6: Chronic inhalation exposure to 24.8 ppm nitrobenzene resulted in an increased incidence of focal pancreatic acinar cell hyperplasia in male F344 rats (Cattley et al. 1994, 1995; CIIT 1993).

Section 2.13: Male F344 rats showed an increase in the incidence of diffuse hyperplasia of the parathyroid gland after exposure to 24.8 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993).

COMMENT 17: Referring to the statements in Section 2.15—Oladele et al. (2020b) evaluated neurobehavioral effects and brain histopathology in Wistar rats given 100 mg/kg/day nitrobenzene by daily gavage for 14 days. The exposed rats exhibited decreased exploratory behavior and increased defecation, and degenerative lesions in the cerebellum, cerebrum, and hippocampus. In addition, the study authors reported increased acetylcholinesterase activity and decreased dopamine levels in the brain (Oladele et al. 2020a)—the Reviewer commented "This paper does not report incidence and severity of histopathologic findings or indicate qualification of observer as a pathologist. The micrographs of "representative" sections lack sufficient quality to demonstrate lesions."

RESPONSE: *It is not unusual for publications such as Oladele et al. (2020b) to provide only qualitative information on pathology findings, and it is relatively rare for published papers to include the qualifications of the pathologist. The text in Section 2.15 was revised to indicate that the finding of degenerative lesions was as reported by the study authors. No other change was made.*

The exposed rats exhibited decreased exploratory behavior and increased defecation, and the study authors reported degenerative lesions in the cerebellum, cerebrum, and hippocampus.

COMMENT 18: Referring to the statement in Section 2.15—In the 14-day gavage study in Wistar rats described above, Oladele et al. (2020a) also showed that nitrobenzene exposure (100 mg/kg/day) increased oxidative stress in the cerebrum, mid brain, and cerebellum, as shown by statistically significant increases in malondialdehyde and hydrogen peroxide levels and decreases in levels of superoxide dismutase, reduced glutathione, and catalase—the Reviewer commented "It is not clear whether oxidative stress was established in this study or if these are secondary effects."

RESPONSE: *No change suggested or implied by the comment. No response needed.*

COMMENT 19: The Reviewer commented "Note that reference Cattley et al (1994) was followed by an Erratum published in 1995. The results and the conclusions were not affected. A copy is attached."

RESPONSE: *The erratum citation was added throughout the profile.*

Comments provided by Peer Reviewer #3:

ATSDR Charge Questions and Responses and Reviewer Comments

Chronic Inhalation MRL

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain. 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. 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 1: Total uncertainty factor seems appropriate.

RESPONSE: *No response needed.*

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

COMMENT 2: No comment.

RESPONSE: *No response needed.*

Unpublished Study

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

COMMENT 3: Numbers were robust and animal care adequate. Upon noting mouse loss due to "animal husbandry procedures", mouse housing was modified (details not provided). Document states that mice and rats were lost at similar rates across all groups and that these losses did not affect data analysis.

RESPONSE: *No response needed.*

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

COMMENT 4: Yes

RESPONSE: *No response needed.*

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

COMMENT 5: Three treatment inhalation concentrations plus HEPA-filtered air controls were performed on both mice and rats. Exposure concentrations were based on an earlier 90-day study.

RESPONSE: *No response needed.*

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

COMMENT 6*:* I believe that the design and reporting were fine. My only issue was that many of the tables between pages 213 and 254, and then again p 444 and after were a bit difficult to read due to the quality of the reproduction.

RESPONSE: *No response needed.*

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

COMMENT 7*:* I have no reason to dispute the conclusions of the report.

RESPONSE: *No response needed.*