

**DISPOSITION OF PEER REVIEW COMMENTS FOR
TOXICOLOGICAL PROFILE FOR
NITRATE AND NITRITE**

Prepared by:

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Prepared for:

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Peer reviewers for the third pre-public comment draft of the Toxicological Profile for Nitrate and Nitrite were:

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Comments that are purely stylistic in nature were addressed at the discretion of ATSDR and do not appear in this formal disposition. Comments that warrant a formal response are listed below.

Comments provided by Reviewer #1:

Reviewer #1 provided a file that contains comments in a summary titled “ATSDR Assessment of Nitrate/Nitrite.” ATSDR has categorized these comments according to page number (e.g., P1), paragraph number (e.g., Para1), and intra-paragraph line number(s) (e.g., L1-3) within this file.

COMMENT: P2 Para1 L2-3: Reviewer #1 stated that the public health statement would benefit from a short discussion of routes of exposure following release of nitrate and nitrite from sources that include historic waste disposal facilities.

RESPONSE: *Routes of exposure were added the Public Health Statement under a section titled “HOW MIGHT I BE EXPOSED TO NITRATE AND NITRITE?”*

COMMENT: P2 Para1 L3-5: Reviewer #1 indicated that it might be beneficial to include advice regarding restriction of unnecessary exposure via drinking water by preventing contamination of wells where consumers are using small supplies.

RESPONSE: *Information regarding restriction of unnecessary exposure via drinking water by preventing contamination of wells where consumers are using small supplies is presented in the Public Health Statement under a section titled “HOW CAN FAMILIES REDUCE THE RISK OF OVEREXPOSURE TO NITRATE AND NITRITE?”*

COMMENT: P2 Para2 L1-2: Reviewer #1 provided a study (Nitrate in drinking water and childhood-onset insulin-dependent diabetes mellitus in Scotland and Central England; DWI 1999) that was not cited in the Toxicological Profile for Nitrate and Nitrite.

RESPONSE: *Relevant study information in this study was added to the profile.*

COMMENT: P2 Para2 L3-6: Reviewer #1 stated: “The coverage of the nitrogen cycle in the body is good but could be better highlighted in the earlier sections because it is important to put nitrate and nitrite into context, since these are not man-made toxic organic chemicals or even on a par with arsenic or fluoride.”

RESPONSE: *Brief statements regarding the nitrogen cycle were added to revised text in Chapters 1 and 2.*

COMMENT: P2 Para2 L6-8: Reviewer #1 stated: “There is increasing evidence that nitrate, through the nitric oxide pathway may have beneficial effects on arterial muscle tone and beetroot juice, which is rich in nitrate, is being taken by top level athletes under scientific supervision.”

RESPONSE: *A statement was added to Section 3.2.2.2 (Cardiovascular Effects) to indicate possible beneficial effects of short-term dietary supplementation of inorganic nitrate.*

COMMENT: P2 Para3 L1-3: Reviewer #1 stated: “The document would benefit from a brief statement at the end of each section relating to a particular endpoint regarding the weight or balance of evidence for and against there being a credible concern.”

RESPONSE: *Statements regarding weight of evidence for carcinogenicity are included in the cancer sections of Chapters 1, 2, and 3. Introductory summary statements regarding quality of evidence for hematological effects and metabolic effects were included in Section 3.2.2.2. Introductory summary statements for endocrine effects and developmental effects were added to Section 3.2.2.2. Other sections in Chapter 3.2 contain limited information and do not appear to necessitate summary statements.*

COMMENT: P2 Para4 L1-2: Reviewer #1 stated: “I have included comments in the text relating to specific items but it is important in a document that is readily available to the lay person to make sure that the wording is absolutely clear.”

RESPONSE: *Specific comments on annotated pages were addressed as noted in responses on the annotated pages.*

COMMENT: P2 Para4 L3-4: Reviewer #1 stated: “The classification of headache as a neurological effect is potentially misleading unless there is clear evidence that it is a direct effect on the nervous system.”

RESPONSE: *This comment was addressed in the comment on annotated pages.*

COMMENT: P2 Para5 L1-2: Reviewer #1 stated: “The differences in metabolism and handling of nitrate and nitrite between different species, particularly between rats and man should, perhaps, be made clear earlier in the document.”

RESPONSE: *This point is made as early as Section 2.3 and does not appear to be necessary in the Public Health Statement.*

COMMENT: P2 Para6 L1 to P3 Para3 L4: Reviewer #1 stated: “While I can understand the use of the Walton studies as a basis for an MRL, this is based on bottle-fed infants but does not really apply to other age groups. While it is protective of all other age groups it will be highly precautionary (conservative) in nature. Even in terms of bottle-fed infants and the Walton study, there are significant uncertainties arising from the good evidence that inflammatory processes, such as in GI infections, also play an important role in the development of methaemoglobinaemia. This is reflected in the data that we have relating to exposure of bottle-fed infants in many parts of the world to water concentrations greater than 44 mg/l nitrate (or 50 mg/litre nitrate) with signs of methaemoglobinaemia. In addition the Walton study only considered nitrate from drinking water for bottle-fed infants and the MRL appears to be intended to cover total exposure. This potentially creates a risk of misunderstanding and misinterpretation, since using this figure would result in a calculated safe level for drinking water that is considerably less than the current EPA standard and other standards and guidelines around the world, even without consideration of exposure from food. In addition the details of the derivation are not properly and clearly explained even in appendix B. I am surprised, given the information provided indicating that the Walton study probably overestimates the formation of methaemoglobinaemia in the absence of concurrent GI infection, that there is no explanation as to why, in spite of this, the Walton study was used in a way in which extremely conservative, probably excessively conservative, values would be derived. 4 mg/kg ingested by a 10 kg

child would be equivalent to drinking less than 1 litre of drinking water at the current EPA standard with no allowance for intake from food. One solution for this, given the difficulties, would be to clearly explain how the MRL was derived and then provide a statement outlining the uncertainties and conservatism. This is a minimum risk level and may be regarded as one below which there is effectively no risk even to the most vulnerable population group.”

RESPONSE: *Presentation of the oral MRLs for nitrate was expanded to note that the MRLs are highly conservative because they were derived using results from a particularly sensitive population exhibiting nitrate-induced methemoglobinemia (infants <3 months of age), and because increased risk of methemoglobinemia in this sensitive population may have been due in part to exposure to contaminants other than nitrate.*

COMMENT: P3 Para4 L1: Reviewer #1 stated: “Much of the comment regarding the MRL for nitrate applies also to the MRL for nitrite.”

RESPONSE: *Presentation of the oral MRLs for nitrite was expanded to note that the MRLs are highly conservative because they were derived using results from a particularly sensitive population exhibiting nitrate-induced methemoglobinemia (infants <3 months of age), and because increased risk of methemoglobinemia in this sensitive population may have been due in part to exposure to contaminants other than nitrate.*

COMMENT: P3 Para5 L1-4: Reviewer #1 stated: “Although it is implicit in the document that nitrate and nitrite act together, i.e. nitrate is converted to nitrite, there is no discussion of possible addition if exposed to both. In view of the highly conservative nature of the proposed MRLs, this is almost certainly of no account. However, it would seem reasonable to at least comment.”

RESPONSE: *A statement was added to Section 2.3 to note that exposure to nitrate and nitrite can occur together, that nitrate and nitrite can be interconverted in the body, and that WHO (2011a, 2011b) includes guidance for combined exposure to nitrate and nitrite in drinking water, which states that the sum of the ratios of the concentration of each to its guideline value should not exceed 1.*

COMMENT: P3 Para6 L10 to P4 Para1 L2: Reviewer #1 stated: “ATSDR has carried out a comprehensive assessment of the literature regarding the potential adverse health consequences of consuming high levels of nitrate and nitrite. However, the data available for deriving suitable MRLs is limited, largely because these substances are part of the natural nitrogen cycle and are not easily studied for toxicity due to the various confounding variables. As a consequence nitrate and nitrite do not fit comfortably within ATSDR’s remit. ATSDR has, therefore, been confronted with the same dilemmas faced by other agencies in the US and around the world. Other agencies have generally considered only one source of exposure and ignored other sources while this is difficult for ATSDR. To address this problem ATSDR have tried to use the existing data for drinking water and bottle-fed infants (formula-fed) as the acknowledged group at highest risk for the most sensitive endpoint methaemoglobinaemia. However, data derived subsequent to the most significant study of Walton shows that there is an important confounding factor in the form of inflammatory processes associated with GI infections that may well have been present in at least some of the subjects studied. Unfortunately the data available do not provide any easy clear cut solutions to this dilemma. The result is that the MRLs are highly conservative. One approach that would potentially help to alleviate some of this conservatism would be to use a smaller body weight in determining the dose per unit body weight to be calculated from the Walton study, reflecting that many of the infants concerned would be quite young. For example using a 5

kg infant drinking 0.75 litres per day would give an intake of 6.6 mg/kg which would round to 7 mg/kg. However, this still leaves something of a problem because this is using the most vulnerable category that are such mostly because of a particular set of circumstances, i.e. bottle-feeding, that sets them apart from the rest of the population.”

RESPONSE: *This comment is a summarization of comments made earlier; no response is necessary.*

COMMENT: P4 Para2 L1-3: Reviewer #1 stated: “To further complicate this WHO, for example, has acknowledged that nitrate and nitrite can be present simultaneously in water and have proposed a formula to take into account their differing potency in determining an additive affect.”

RESPONSE: *This information was added to Section 2.3.*

COMMENT: P4 Para3 L1-3: Reviewer #1 stated: “WHO and Health Canada have both identified interference with iodine uptake as a possible concern but there is inadequate evidence to determine whether this really is of relevance to humans and certainly inadequate data to derive an MRL based on this endpoint.”

RESPONSE: *The toxicological profile for nitrate and nitrite includes available information regarding nitrate and potential inhibition of iodine uptake and notes limitations of the studies.*

Reviewer #1 submitted an electronic file of draft 3 of the Toxicological Profile for Nitrate and Nitrite using tracked changes and embedded comments. Tracked changes and comments that were of sufficient nature to warrant a formal response are itemized below and identified by page (P) and line (L) on which they are found in the file submitted by Reviewer #1. Note: Page and line numbers in this file do not correspond to the page and line numbers in the file of draft 3 of the Toxicological Profile for Nitrate and Nitrite that was provided to Reviewer #1 due to the use of tracked changes, which included suggested additions and deletions.

COMMENT: P3 (How Nitrate/Nitrite Can Affect Your Health; How nitrate/nitrite enters your body; Soil): Reviewer #1 stated “There really isn’t any solid evidence for absorption through skin from soil and this seems very unlikely, however pica may make a small contribution in young children.”

RESPONSE: *The text was revised to state “However, nitrate or nitrite in soil could enter the body of young children if they put soil containing nitrate or nitrite in the mouth.”*

COMMENT: P3 (How Nitrate/Nitrite Can Affect Your Health; How nitrate/nitrite enters your body; Other): Reviewer #1 suggested that the statement “Exposure to nitrate may also occur from internal production” should be revised to read: “Nitrate may also be present in the body from internal production.”

RESPONSE: *The sentence was revised to state “Nitrate is also present in the body from internal production.”*

COMMENT: P4 (How Nitrate/Nitrite Can Affect Your Health; What happens to nitrate/nitrite in your body): Reviewer #1 suggested adding the statement: “There is no difference chemically between nitrate or nitrite formed in the body and nitrate and nitrite from external sources.”

RESPONSE: *The following statement was added: “Nitrate and nitrite are widely distributed in the body. Nitrate and nitrite that enter your body are no different chemically than nitrate and nitrite produced inside your body.”*

COMMENT: P4 (How Nitrate/Nitrite Can Affect Your Health; Short-term exposure effects): Reviewer #1 suggested that the sentence “The cause of methemoglobinemia in many of these infants may gastroenteritis from other contaminants in drinking water or from other sources not related to nitrate” be revised to state “The cause of methemoglobinemia in many of these infants may be gastroenteritis from bacteria or viruses in drinking water or from other sources not related to nitrate.”

RESPONSE: *The requested change was made.*

COMMENT: P4 (How Nitrate/Nitrite Can Affect Your Health; Nitrate/Nitrite and Cancer): Reviewer #1 suggested that the sentence “Cancer may be the result of reactions....” be changed to read “Cancer could result from reactions....”

RESPONSE: *The suggested revision was made.*

COMMENT: P5 (Children and Nitrate/Nitrite; Overview): Reviewer #1 stated: “Fundamentally this section relates to infants and young children, not older children and certainly not up to 18 years of age.”

RESPONSE: *The Overview text is common to ATSDR toxicological profiles and is not specific to the Toxicological Profile for Nitrate and Nitrite.*

COMMENT: P6 (How Can Families Reduce the Risk of Exposure to Nitrate/Nitrite; Food): Reviewer #1 suggested that a statement be added to note that the information regarding food may particularly apply to children.

RESPONSE: *A statement “This consideration is particularly relevant to infants and small children” was added.*

COMMENT: P6 (How Can Families Reduce the Risk of Exposure to Nitrate/Nitrite; Drinking water): Reviewer #1 suggested that the statement “Don’t drink water containing high levels of nitrate or nitrite” should include reference to concentrations above the standards for drinking water.

RESPONSE: *The statement was revised to read: “Don’t drink water containing levels of nitrate or nitrite higher than guideline levels for drinking water.”*

COMMENT: P6 (How Can Families Reduce the Risk of Exposure to Nitrate/Nitrite; Contaminated groundwater or soil): Reviewer #1 stated: “Will people make a distinction between groundwater that

they drink and drinking water? The inclusion of soil seems to unnecessarily dilute the impact of real sources of exposure as it will be very minor even for children exhibiting pica.”

RESPONSE: *The entire entry was deleted.*

COMMENT: P6 (Medical Tests to Determine Nitrate/Nitrite Exposure; Nitrate/Nitrite can be measured in blood and urine): Reviewer #1 suggested revising the sentence “However, these tests cannot tell whether the high methemoglobin levels were caused by nitrate and nitrite or by some other substance” to “However, these tests cannot tell whether the high methemoglobin levels were caused by nitrate and nitrite or by some other substance or disease.”

RESPONSE: *The suggested change was made.*

COMMENT: P10 L20-22: Reviewer #1 stated that the WHO guideline values are 50 mg/L (nitrate) and 3.0 mg/L (nitrite). The Reviewer also noted that WHO suggested that because nitrate and nitrite have similar modes of action in causing methemoglobinaemia, they should be considered together when both are present in drinking water so that the sum of the concentration of nitrate and nitrite divided by the guideline for each should be no greater than 1.

RESPONSE: *The statement regarding MCLs for nitrate and nitrite in drinking water was removed from Section 2.1. The correct MCL values for nitrate and nitrite in drinking water appear in Table 8-1, along with the statement of WHO regarding guidelines when both nitrate and nitrite are present.*

COMMENT: P11 L8-10: Reviewer #1 stated that there is no evidence that neurological symptoms are direct effects of nitrate on the nervous system and suggested that it is secondary to reduced oxygen transfer.

RESPONSE: *The sentence was revised to read: “Symptoms involving the central nervous system...” The statement was not intended to suggest that nitrate acts directly on central nervous system function. This section simply presents information regarding clinical signs associated with various levels of methemoglobin.*

COMMENT: P11 L13-16: Reviewer #1 suggested that the statement regarding differences between fetal and adult hemoglobin with respect to oxygenation should be expanded with regards to the impact on the child and what it means for health.

RESPONSE: *The statement in question was revised to read “Furthermore, due to differences in the oxygen carrying capacity between fetal hemoglobin and adult hemoglobin (which replaces fetal hemoglobin during the first year of postnatal life), cyanosis in young infants with mostly fetal hemoglobin may not be detected at methemoglobin levels eliciting clinical cyanosis in older infants with mostly adult hemoglobin (Steinhorn 2008).”*

COMMENT: P12 L23-28: Regarding evidence of associations between elevated levels of nitrate in drinking water and/or nitrate-rich diets and signs of thyroid dysfunction, Reviewer #1 stated that epidemiological and clinical studies (Blount et al. 2009; Hunault et al. 2007) found no association.

RESPONSE: *The following sentence was added: “Hunault et al. (2007) found no evidence for nitrate-induced effects on thyroid function in adults ingesting 15 mg sodium nitrate/kg (3 times the acceptable daily intake of nitrate) for 38 days.” The report of Blount et al. (2009) does not include information regarding associations between nitrate intake and thyroid function.*

COMMENT: P13 L27 to P14 L7: Reviewer #1 suggested adding the following statement to the end of the discussion regarding nitrate and nitrite in drinking water and/or food sources and risk of type 1 diabetes: “Subsequently Parslow et al repeated this study to correct a number of problems with the study and found nothing (See attached report from the UK Drinking Water Inspectorate).”

RESPONSE: *A statement was added regarding the lack of evidence for an association between childhood type 1 diabetes and nitrate in the drinking water in a subsequent study (DWI 1999).*

COMMENT: P14 L21-27: Reviewer #1 suggested revising the last sentence in the Mahlberg et al. (1978) study summary: “The nitrate-exposed patients exhibited an earlier mean age at hospitalization for hypertension” by adding “although the significance of this finding is uncertain since the number of affected individuals was lower than the control population.”

RESPONSE: *The suggested addition was made.*

COMMENT: P14 L29 to P15 L3: Regarding the summary of the Kortboyer et al. (1997b) study, Reviewer #1 suggested that the effect on blood pressure may be considered beneficial but some additional discussion of the effects of nitrate on blood pressure and arterial muscle-tone would be helpful.

RESPONSE: *A statement was added regarding the beneficial effects of short-term nitrate supplementation on cardiovascular health and precautionary statement regarding benefits of long-term nitrate supplementation.*

COMMENT: P15 L30 to P16 L2: Reviewer #1 asked whether there is any evidence of the reported neurological symptoms being direct neurological effects or secondary to vascular effects.

RESPONSE: *A statement was added to note that observed neurological effects may be the result of decreased oxygen-carrying capacity.*

COMMENT: P17 L11-12: Regarding the sentence that states: “Mechanistically, this outcome is consistent with nitrite and being a reactive intermediate in the cancer mode of action of nitrate...”, Reviewer #1 stated the following: “The term reactive intermediate does not normally apply to something like nitrite, which needs to further react with amines or amides outside the cell to produce other substances that then go on to form potentially active substances, and implies something different to what actually happens.”

RESPONSE: *The word “reactive” was deleted.*

COMMENT: P17 L21-22: Regarding the sentence that states: “One potentially important class of confounders is anti-oxidants that can interfere with nitrosation of dietary amines and, thereby, the mode

of carcinogenicity of nitrite, and may also interfere with other carcinogenic process that involve reactive intermediates,” Reviewer #1 stated: “The term interferes with is not quite right here and it is not nitrite that is carcinogenic.”

RESPONSE: *The sentence was revised to read: “... antioxidants that can influence the degree of nitrosation of dietary amines and, thereby, the cancer risk from exposure to nitrate or nitrite.”*

COMMENT: P27 L1-12: Regarding derivation of the oral MRLs for nitrate, Reviewer #1 stated that this section needs a clear statement as to how the MRLs were derived from the drinking water concentration of 44 mg/L and explanation for assumptions that were made. Reviewer #1 further suggested that it be made clear that the MRLs are considered to be highly conservative.

RESPONSE: *Information was added regarding calculation of a dose at the NOAEL of 44 mg/L. The values used for drinking water consumption and body weight are explained in detail in Appendix A to which the reader is referred for additional details. A statement was added to note that the MRLs are considered to be highly conservative.*

COMMENT: P28 L8-18: Reviewer #1 stated that the derivation of the oral MRLs for nitrite are not explained clearly and transparently enough.

RESPONSE: *The derivation of oral MRLs for nitrite appear to be adequately explained in Section 2.2 and in Appendix A. No change was made.*

Comments provided by Reviewer #2:

Reviewer #2 provided comments in a Word file in which comments were associated with consecutive page numbers in the Word file of the draft 3 Toxicological Profile for Nitrate/Nitrite provided to the Reviewer, not the printed page numbers in the Toxicological Profile. For example, page 3 of the draft 3 Toxicological Profile for Nitrate/Nitrite was listed as page number 23 in the Word file provided by Reviewer #2. This formal disposition identifies Reviewer #2 comments by printed page number(s) within the draft 3 Toxicological Profile for Nitrate/Nitrite that was provided to the Reviewer for comments.

COMMENT: P3 (Where is nitrate/nitrite found?; Soil): Reviewer #2 suggested adding another citation: Bronson, K.F. 2008. Forms of Inorganic Nitrogen in Soil IN J.S. Schepers and W. R. Raun, Eds. Nitrogen in Agricultural Systems, Agronomy Monograph 49, American Society of Agronomy.

RESPONSE: *The suggested addition was made.*

COMMENT: P9 L17-20: Reviewer #2 stated: “Need to clarify that significant uptake of nitrate occurs in all plants; internal storage of nitrate, rather than metabolic conversion to ammonium and amino acids, can occur in some plants, especially leafy vegetables.”

RESPONSE: *The suggested addition was made.*

COMMENT: P129 L1-11: Reviewer #2 stated: “This paragraph needs revision to reflect the reality that the majority of nitrate derived from fertilizers in the environment does not originate as a fertilizer in the nitrate form; it is converted to nitrate from the biological process of hydrolysis of urea to ammonium and the microbial process of nitrification from ammonium to nitrate (Kissel et al., 2008).”

RESPONSE: *The paragraph on P 129 L22-32 was revised to state: “A major source of anthropogenic nitrate and nitrite is artificial fertilizers (WHO 1978). The majority of nitrate in the environment, derived from fertilizers, does not solely originate from nitrate containing fertilizers; it also comes from ammonium and urea fertilizers. Nitrate from ammonium and urea fertilizers is produced through biological processes involving hydrolysis of urea to ammonium and ammonium nitrification (Kissel et al. 2008). Approximately 11.5 million tons of nitrogen are applied yearly (as of 1994) in the United States as fertilizer in agricultural areas (Nolan et al. 1997). Ammonium, calcium, potassium, and sodium salts are all used in commercial fertilizers compounds (IARC 2010; WHO 2011b). The most common nitrite salt, sodium nitrite, is produced commercially via the reaction of nitrogen oxides with sodium carbonate or sodium hydroxide solution, typically at a pH higher than 8 (Hammerl and Klapotke 2006). In 2004, global production of sodium nitrate was about 63 kilotons (IARC 2010). Ammonium nitrate is manufactured through the reaction of nitric acid and ammonium (HSDB 2007). Global production of ammonium nitrate in 2002 was reported at 13,608 kilotons (IARC 2010). Between 1998 and 1999, 90 kilotons of Canadian fertilizers were nitrate compounds: 82% as ammonium nitrate and the remaining 18% from calcium nitrate, calcium ammonium nitrate, and potassium nitrate (Environment Canada 2012).” The paragraph on P 129 L1-11 was moved to follow the revised paragraph.*

COMMENT: P129 L23: Reviewer #2 noted that nitrogen fertilizer use in U.S. data has been updated to 2012 (13.5 million tons N). The source for this information was cited by the Reviewer as: The Fertilizer Institute. <http://www.tfi.org/statistics/fertilizer-use> (accessed 10/23/14).

RESPONSE: *The updated information was added to Chapter 5.*

COMMENT: P130: Reviewer #2 questioned the value of Table 5-3 and noted that ammonium nitrate is an insignificant source of nitrate as a fertilizer in the United States or worldwide. The Reviewer suggested that Table 5-3 be accompanied by tables documenting trends in production of ammonia and urea.

RESPONSE: *In revised tables of Chapter 5, Table 5-4 (formerly Table 5-3) presents data regarding production of ammonium nitrate by the U.S. chemical industry. A new table (Table 5-5) presents data regarding production of ammonia by the U.S. chemical industry.*

COMMENT: P131-133: Reviewer #2 stated that import/export of primary N fertilizers ammonia and urea should be documented in Table 5-4.

RESPONSE: *Table 5-7 (formerly Table 5-4) was revised to include data for urea and ammonia.*

COMMENT: P136-139: Reviewer #2 questioned the relevance of Figures 6-1 to 6-4, noted that the type of contamination is not given, occurrence is low, and the information is dated.

RESPONSE: *Figures 6-1 to 6-4 were retained, although it is noted that the HazDat database is no longer updated (as of 2008).*

COMMENT: P147 L20-32: Reviewer #2 stated that Section 6.2.3 documents release of nitrate- and nitrite-containing compounds from manufacturing and processing facilities; however, the totals provided here are insignificant compared to the 13.5 million tons of N added annually to soils as fertilizer in 2012, the vast majority of which converted to nitrate once applied to soil.

RESPONSE: *Available TRI data are used here. The section was revised to include information that inorganic nitrogen fertilizers and nitrification of animal waste are the principal sources of nitrate in the environment. Therefore, it should be noted that the totals provided are insignificant compared to the 13.5 million tons of nitrogen added to soils as fertilizer in 2012 (www.tfi.org.....pdf). TRI data for ammonia were added to the section.*

COMMENT: P148 L6: Reviewer #2 requested the addition of clarification that plants take up both ammonium and nitrate-N, citing Bronson (2008; Forms of inorganic nitrogen in soil. In: J.S. Schepers and W.R. Raun, Eds. Nitrogen in agricultural systems, Agronomy Monograph 49, American Society of Agronomy) as a source for this information.

RESPONSE: *The requested clarification was made, but cited to a different source (Cornell 2009).*

COMMENT: P148 L25: Reviewer #2 suggested that the sentence “Nitrate does not adsorb onto soil; therefore, leaching of excess soil nitrate into oceans ...” be revised to state “Sorption of anions such as nitrate is insignificant in most soils; therefore, leaching of excess soil nitrate into oceans,”.

RESPONSE: *The requested change was made.*

COMMENT: P148 L32-33: Reviewer #2 stated: “While the citation of an example of nitrate leaching from a European study is perhaps useful, the absence of any literature citations of nitrate contamination of groundwater in the US is puzzling. A perhaps useful case study is Nebraska’s Central Platte River Valley. Numerous studies and publications have documented the steady increase of nitrate in groundwater in this regions starting in the 1960s. Subsequent studies have documented the impacts of improved agricultural management practices on mitigating groundwater nitrate levels. Following are selected publications which may be useful:

Schepers, J.S., M.G. Moravek, E.E. Alberts, and K.D. Frank. 1991. Maize production impacts on groundwater quality. *J. Environ. Qual.* 20:12-16

Spalding, R.F., and M.E. Exner. 1993. Occurrence of nitrate in groundwater – a review. *J. Environ. Qual.* 22:392-402.

Spalding, R.F., D.G. Watts, J.S. Schepers, M.E. Burbach, M.E. Exner, R.J. Poreda, and G.E. Martin. 2001. Controlling nitrate leaching in irrigated agriculture. *J. Environ. Qual* 30:1184-1194

Exner, M.E., H. Perea-Estrada, and R.F. Spalding. 2010. Long-term response of groundwater nitrate concentrations to management regulations in Nebraska’s Central Platte Valley. *The Scientific World Journal* 10:286-297.

Ferguson, R.B. 2014. Groundwater quality and nitrogen use efficiency in Nebraska’s Central Platte River Valley. *J. Environ. Qual.* doi:10.2134/jeq2014.02.0085; posted 28 Aug. 2014.”

RESPONSE: *Information from Schepers et al. (1991) and Exner et al. (2010) was added.*

COMMENT: P172: Reviewer #2 stated: “Commonly used, standard methods for nitrate and nitrite analysis in soil are not listed in this table. A primary reference for analytical methodology for nitrate and nitrite in soils is listed below. Multiple analytical methods are listed, but the most commonly used is colorimetric determination following cadmium reduction in a continuous flow analyzer.

Mulvaney, R.L. 1996. Nitrogen – Inorganic Forms. IN *Methods of Soil Analysis, Part 3: Chemical Methods.* Book Series No. 5. Soil Science Society of America and American Society of Agronomy.”

RESPONSE: *Relevant information from the suggested reference (Mulvaney 1996) was added to Section 7.2 and Table 7-2.*

Comments provided by Reviewer #3:

Reviewer #3 submitted an electronic file that contains comments related to charge questions provided by ATSDR.

CHAPTER 1

COMMENT: Charge question: *Does the chapter present the important information in a non-technical style suitable for the average citizen? If not, suggest alternative wording.* Reviewer #3 commented: “The style is non-technical, but some of the wording is too technical for a lay language summary. Specific examples and proposed alternatives are identified below. As a general comment, Chapter 1 treats nitrate and nitrite separately in some sections providing information on each. In other sections, only information on nitrate is provided with no mention of nitrite, and in others the terms are used almost interchangeably (e.g., reference to “nitrate/nitrite”). I think that there is great potential for confusion of the lay reader on the extent to which these are separate substances.”

RESPONSE: *Response to the statement regarding specific examples is covered in specific examples and alternatives proposed by the Reviewer. The format of Chapter 1 was completely revised; in the process of reorganization, the term “nitrate/nitrite” was eliminated. Information relevant to nitrate and/or nitrite should be clear to the reader in the revised version of Chapter 1.*

COMMENT: Charge question: *In your opinion, do the answers to the questions adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them.* Reviewer #3 commented: “In general, the responses in each of the subject areas should adequately address concerns by the lay public. Consider adding a question about interactions between nitrate/nitrate and other chemicals. The public is increasingly aware that chemical exposures do not occur in isolation and are interested in whether there are important chemical interactions that might occur and whether these have been taken into consideration. There is a section in the profile that deals with this (Section 3.9), and whatever information can be brought forward in a lay summary might be good to add.”

RESPONSE: *Interactions between nitrate and/or nitrite and other substances mainly involves the formation of potentially carcinogenic substances from reactions that involve nitrite and nitrosating compounds. Information regarding such interactions is included in the revised section of Chapter 1 titled “HOW CAN NITRATE AND NITRITE AFFECT MY HEALTH?”.*

COMMENT: “The response to the question ‘Why a nitrate/nitrite release can be harmful’ reads more like ‘Why a nitrate/nitrite release may not be harmful.’ Somewhere in the answer it should clearly acknowledge that a release has the potential to produce adverse effects in an exposed individual and then provide the caveats in the current response.”

RESPONSE: *This section is not included in the revised format of Chapter 1.*

COMMENT: “In the response to the question ‘Where is nitrate/nitrite found?’ the table of possible sources has no information for nitrite in surface water, drinking water, and groundwater or soil, leaving the reader to wonder where it is found there.”

RESPONSE: *The revised section is titled “WHERE ARE NITRATE AND NITRITE FOUND” and includes information on nitrite as well as nitrate.*

COMMENT: “In the response to the statement ‘How nitrate/nitrite enters your body’ the table has seemingly contradictory statements about soil. The first sentence indicates that nitrate/nitrite entering your body from soil is unlikely, and the next sentence says that they could enter your soil if you get soil on your skin or in your mouth. Those seem like pretty common exposure pathways to me. I suggest deleting the first sentence and ‘However’ at the beginning of the second sentence in this table entry.”

RESPONSE: *The section was renamed “HOW CAN NITRATE AND NITRITE ENTER AND LEAVE MY BODY?” and includes a statement that neither nitrate nor nitrite are likely to enter your body from soil, with the exception of young children who might put soil containing nitrate or nitrite in the mouth.*

COMMENT: “Also, in the response to the statement ‘How nitrate/nitrite enters your body’ the table entry for ‘Other’ will not be understood by the public. I suggest just saying something like ‘Some nitrate is produced in the body as part of normal metabolism.’”

RESPONSE: *The revised format for this section does not include a section titled “Other”. A statement regarding production of nitrate and nitrite inside the body is included in the revised section.*

COMMENT: “In the answer to the statement ‘How nitrate/nitrite leaves your body,’ everything but the first sentence would be more appropriate in the preceding section ‘What happens to nitrate/nitrite in your body.’”

RESPONSE: *The revised format does not include subdivisions for the section titled “HOW CAN NITRATE AND NITRITE ENTER AND LEAVE MY BODY?”.*

COMMENT: “The answer to the statement ‘Short-term exposure effects,’ illustrates the potential confusion created by ambiguity in distinguishing between nitrate and nitrite. The first half of the narrative talks about nitrite, and in the middle it switches to nitrate, seemingly as part of the same narrative regarding methemoglobin effects. Is this intentional? If so, the distinction between nitrite and nitrate regarding this effect is unclear.”

RESPONSE: *This section was reorganized to present information regarding nitrate, followed by information regarding nitrite.*

COMMENT: “The response to the statement ‘Exposure effects for children generally’ includes the statement ‘Young infants ... appear to be particularly sensitive to the effects of nitrate on methemoglobin levels ...’ What about nitrite?”

RESPONSE: *The statement was revised to read “Small infants (<6 months of age) appeared to be particularly sensitive to the effects of nitrite on hemoglobin after consuming formula prepared with water that contained nitrate at levels higher than recommended limits; some of these infants died.”*

COMMENT: Charge question: *Are scientific terms used that are too technical or that require additional explanation? Please note such terms and suggest alternate wording.* Reviewer #3 commented: “Overview, second section (‘Nitrate/nitrite at hazardous waste sites’), fourth sentence: Replace ‘Specific salts ...’ with ‘Specific forms of nitrate and/or nitrite ..’”

RESPONSE: *The suggested rewording was incorporated into the revised format for the appropriate section of Chapter 1.*

COMMENT: “Overview, fourth section (‘What is nitrate/nitrite?’), second sentence: Replace ‘They typically exist in the environment as highly water soluble salts, associated with metal cations such as sodium and potassium.’ with ‘They typically exist in the environment in highly water soluble forms in association with other ions such as sodium and potassium.’”

RESPONSE: *The suggested rewording was incorporated into the revised format for the appropriate section of Chapter 1.*

COMMENT: “Overview, fourth section (‘What is nitrate/nitrite?’), last sentence: Replace ‘biotic processes’ with “biological processes involving plants, microbes, and others.’”

RESPONSE: *The suggested rewording was incorporated into the revised format for the appropriate section of Chapter 1.*

COMMENT: “Overview, sixth section (‘Where is nitrate/nitrite found?’), second sentence: Replace ‘debris nitrification’ with ‘decomposition’”

RESPONSE: *The suggested rewording was incorporated into the revised format for the appropriate section of Chapter 1.*

CHAPTER 2

COMMENT: Charge question: *Do you agree with those effects known to occur in humans as reported in the text? If not, provide a copy of additional references you would cite and indicate where (in the text) these references should be included.* Reviewer #3 commented: “I agree with the effects known to occur in humans as reported in the text and have no additional references to add.”

RESPONSE: *No response necessary.*

COMMENT: Charge 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.* Reviewer #3 commented: “There are no effects observed only in animals that are likely to be a concern to humans. The only effects of potential interest in this regard are EEG abnormalities and behavioral effects from sodium nitrite in drinking water given to rats and mice, but I agree that the information available on these effects is too limited at present to them as effects of concern for humans.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Have exposure conditions been adequately described? If you do not agree, please explain.* Reviewer #3 commented: “For the purposes of this chapter, the exposure conditions are adequately described.”

RESPONSE: *No response necessary.*

CHAPTER 3

Section 3.2

Human studies

COMMENT: Charge 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)? If not, were the major limitations of the studies sufficiently described in the text without providing detailed discussions. If study limitations were not adequately addressed, please suggest appropriate changes.* Reviewer #3 commented: “Human studies of adequate design are described in the text. The primary effects of interest do not require prolonged exposure, and most of the studies summarized were of adequate duration to provide information of value. By and large, potential confounding factors were adequately addressed in study designs. Limitations in study design and interpretation were noted as appropriate.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)? Please suggest appropriate changes.* Reviewer #3 commented: “Conclusions by the study authors were accurately reflected in the profile and, for the most part, were appropriate. When they were not, this was noted in the profile. I do not disagree with any of these assessments.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.* Reviewer #3 commented: “The NOAELs and LOAELs were appropriately identified for the studies. When NOAELs and LOAELs could not be identified, the reason was adequately explained in the profile.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly?* Reviewer #3 commented: “In general, statistical methods used in individual studies were not presented in the profile. An assessment of the appropriateness of statistical methods in all of the studies cited in the profile is beyond the scope of this review.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are you aware of other studies which may be more 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.* Reviewer #3 commented: “I am not aware of any studies that would be more important in evaluating the toxicity of nitrate/nitrite than those cited in the profile.”

RESPONSE: *No response necessary.*

Animal studies

COMMENT: Charge 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.* Reviewer #3 commented: “Assessment of the potential toxicity of nitrate/nitrite is based primarily on studies in humans. Compared with most profiles, there is much less emphasis on studies in animals. The most important contribution of animal studies is in the assessment of potential carcinogenesis. For these studies, the animal studies are generally well conducted, with sufficient animals and numbers of dose groups that are at least standard for carcinogenesis bioassays.”

RESPONSE: *No response necessary.*

COMMENT: Charge 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?* Reviewer #3 commented: “Most of the animal studies were conducted in rats and mice. These are standard models and appropriate for toxicological endpoints of interest for nitrate/nitrite, including hematological effects (methemoglobinemia), thyroid effects, cancer, etc. Limitations in the use of animal models for nitrate/nitrite effects is discussed briefly (e.g., Section 3.5.2 Animal-to-Human Extrapolations), but probably not often enough in the profile. Expanded (or at least more prominent) treatment of this subject would help the reader understand why the presentation of animal data is not more extensive than it is.”

RESPONSE: *All relevant toxicity studies in animals were included in the draft toxicological profile for nitrate and nitrite. Study limitations regarding species differences mainly concern toxicokinetic differences that limit extrapolation of effect levels in animals to those in humans. It does not appear necessary to make this point in sections other than Section 3.5.2.*

COMMENT: Charge question: *Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?* Reviewer #3 commented: “Conclusions by the study authors were accurately reflected in the profile and, for the most part, were appropriate. When they were not, this was noted in the profile. I do not disagree with any of these assessments.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies? If not, please explain.* Reviewer #3 commented: “The NOAELs and LOAELs were appropriately identified for the studies. When NOAELs and LOAELs could not be identified, the reason was adequately explained in the profile.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *If appropriate, is there a discussion of the toxicities of the various forms of the substance? If not, please give examples of toxicological effects that might be important for forms of the substance.* Reviewer #3 commented: “The form of the substance (nitrate versus nitrite) is important toxicologically, and this is explained well in the profile. However, the discussion of effects of nitrate and nitrite observed in studies are heavily intermingled in the profile, making it difficult sometimes for the reader to understand what distinction should be made between these two forms.”

RESPONSE: *Where possible, text was rearranged or revised to enhance distinction between nitrate and nitrite. However, endogenous interconversion between nitrate and nitrite precludes making a clear distinction in some sections.*

COMMENT: Charge question: *Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly?* Reviewer #3 commented: “In general, statistical methods used in individual studies were not presented in the profile. An assessment of the appropriateness of statistical methods in all of the studies cited in the profile is beyond the scope of this review.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are you aware of other studies that may be important in evaluating the toxicity of the substance? If you are citing a new reference, please provide a copy and indicate where (in the text) it should be included.* Reviewer #3 commented: “I am not aware of any other animal studies that would be important in evaluating the toxicity of nitrate and nitrite.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are the LSE tables and figures complete and self-explanatory? Does the “Users Guide” explain clearly how to use them? Are exposure levels (units, dose) accurately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the “User’s Guide.”* Reviewer #3 commented: “The LSE tables and figures are a familiar fixture of these profiles and an effective means to facilitate comparison of effects and doses among various relevant studies. The “User’s Guide” is clear and the exposure levels are accurate and appropriate. I have no suggestions for improvement.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Do you agree with the categorization of “less serious” or “serious” for the effects for the effects cited in the LSE tables?* Reviewer #3 commented: “I agree with the categorization of the “less serious” and “serious” for the effects cited.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?* Reviewer #3 commented: “The oral MRL for nitrate appears logical and justified. The oral MRL for nitrite, however, is tenuous. It is derived based upon an extrapolation of the nitrite dose estimated to result from ingestion of nitrate at its NOAEL reduced by an additional modifying factor of 2. The extrapolation assumes that 5% of ingested nitrate is reduced to nitrite by bacteria in saliva, as has been demonstrated in adults. However, as the profile explains elsewhere, this isn’t relevant for infants who have limited nitrate reducing bacteria in saliva, but can have bacterial colonization in the stomach [unlike adults]. So the extrapolation is really a guess. The modifying factor in effect assumes that the conversion could be as much as 10%, but there are no data that could be used to defend this as conservative. Unfortunately, I don’t have a solution to this problem to suggest. Studies in humans exposed directly to nitrite are too few and limited to be of much value, and differences in nitrate-nitrite disposition between animals and humans make using animal data problematic. I recognize that the proposed MRL is identical to the U.S. EPA oral RfD for nitrite, and there is some reassurance in that I suppose, but the technical basis for both is not strong.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Have the major limitations of the studies been adequately and accurately discussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?* Reviewer #3 commented: “Limitations in studies are presented in supporting documentation but not extensively discussed in the main profile, except for some of the more important studies. Presenting more critical assessment in the body of the profile would help the reader more easily weigh the value of the information (rather than having to go through the supporting tables), but would create a bulky document. Overall, a reasonable balance is struck.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?* Reviewer #3 commented: “The critical effect is clearly relevant to human health, as well documented by numerous clinical studies and case reports. The effect is supported by studies in animals, but the number and types of human data available make the support from animal studies less important than for chemicals with less human data.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Have “bottom-line” statements been made regarding the relevance of the endpoint for human health?* Reviewer #3 commented: “The relevance to human health comes through clearly from the presentation of the studies, although I could not identify specific “bottom line” statements in this section.”

RESPONSE: *Statements regarding weight of evidence for carcinogenicity are included in the cancer sections of Chapters 1, 2, and 3. Introductory summary statements regarding quality of evidence for hematological effects and metabolic effects were included in Section 3.2.2.2. Introductory summary statements for endocrine effects and developmental effects were added to Section 3.2.2.2. Other sections in Chapter 3.2 contain limited information and do not appear to necessitate summary statements.*

COMMENT: Charge question: *Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided or other data provided to you but not presented in the text.* Reviewer #3 commented: "I agree with the conclusions given the overall database."

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.* Reviewer #3 commented: "Given that the predominant source of exposure (dose) for the human studies is from food and water, the attention to dose-response and the ways of addressing dose-response relationships are appropriate. As noted elsewhere, there is less emphasis on animal data as being of limited relevance to effects in humans."

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.* Reviewer #3 commented: "Where appropriate (e.g., in the discussion of thyroid effects), the animal data have been used to draw support for human effects. In these circumstances the support is valid."

RESPONSE: *No response necessary.*

Section 3.4 Toxicokinetics

COMMENT: Charge question: *Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.* Reviewer #3 commented: "The discussion of absorption, distribution, metabolism and excretion is sufficiently detailed to give an adequate overview of the subject. Figure 3-2 is very helpful in understanding the complex disposition of nitrate and nitrite. The text that accompanies it is also helpful, but is without references to support the statements about the various steps. Similar statements appear in other sections of the document with citations, but the reader who wants a focused discussion of absorption, distribution, metabolism and excretion shouldn't have to scour the rest of the document to find those citations. I suggest that they be added here."

RESPONSE: *The introduction to Section 3.4 (Toxicokinetics) notes that information in this section derives from recent comprehensive reviews, which are cited in a string. Citing individual studies for each topic in the toxicokinetics section would be laborious and of questionable value to the reader. The reviews are publicly available and should be consulted for additional information and clarification.*

COMMENT: Charge question: *Have the major organs, tissues, etc. in which the substance is stored been identified? If not, suggest ways to improve the text.* Reviewer #3 commented: “Nitrate/nitrite does not undergo substantial storage in the body, so this is not an issue for this specific substance.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Have all applicable metabolic parameters been presented? Have all available pharmacokinetic/pharmacodynamics models and supporting data been presented? If not, please explain.* Reviewer #3 commented: “The PBPK model of Zellmaker et al. (1996, 2010) is described in some detail, including the structure of the model, input parameters and parameter values. Sufficient description is provided so that the reader can determine which of the distribution and metabolic processes relevant to nitrate and nitrite are included in the model. Steps to validate the model are also described. Overall, the discussion of this model serves the purpose of this profile well. I am not aware of any other pharmacokinetic models for nitrate/nitrite.”

RESPONSE: *No response necessary.*

COMMENT: “The text notes that some plasma nitrate may be reduced to nitrite in selected tissues (pg 82, lines 6-7). This pathway is not shown in Figure 3-2 and should perhaps be added.”

RESPONSE: *The statement “some plasma nitrate may be reduced to nitrite in selected tissues” was replaced by the following statement later in the same paragraph: “In vitro results using selected rat and mouse tissues and human liver tissue suggest a possible metabolic pathway whereby some plasma nitrate could be reduced to nitrite by enzymes such as xanthine oxidase (Jansson et al. 2008). This potential metabolic pathway was not added to Figure 3-2.*

COMMENT: “As an additional note, the introductory material for Section 3.4.1 provides a general description of PBPK models, which concludes by stating that if such a model exists for nitrate/nitrite, it will be presented below (see page 85, lines 4-6). There is no mystery about whether a model exists—it is discussed immediately afterward. I suggest deleting the text on page 85 (lines 4-6) as superfluous.”

RESPONSE: *This statement is common to all ATSDR toxicological profiles. The inclusion of this statement for the toxicological profile for nitrate and nitrite appears appropriate even though it is obvious that a PBPK model exists.*

COMMENT: Charge question: *Is there adequate discussion of the differences in toxicokinetics between humans and animals? What other observations should be made?* Reviewer #3 commented: “There is virtually no discussion of the toxicokinetics of nitrate/nitrite in animals. For most substances, this omission would be a problem, but the focus of the nitrate/nitrite profile is heavily on information from humans. I don’t object to leaving out toxicokinetic information from animals, but the reason should be clearly explained (see comment below).”

RESPONSE: *Rationale for concentrating on human toxicokinetics data was added to Section 3.5.3.*

CHARGE QUESTION: *If applicable, is there a discussion of the toxicokinetics of different forms of the substance (e.g., inorganic vs. organic mercury)?* Reviewer #3 commented: “The discussion includes

both forms of interest for this profile – nitrate and nitrite. The interrelationships of these two forms in terms of the disposition of ingested nitrate and nitrite is fully addressed.”

RESPONSE: *No response necessary.*

Section 3.5 Mechanisms of Action

COMMENT: Charge question: *Have all possible mechanisms of action been discussed? If not, please explain.* Reviewer #3 commented: “Mechanisms of action regarding methemoglobinemia, possible thyroid effects, and possible carcinogenic effects are each covered briefly but adequately. There are no additional mechanisms of action for toxicity that need to be included.”

RESPONSE: *No response necessary.*

COMMENT: “I note that in this discussion of pharmacokinetic mechanisms, the concentration of nitrate in the salivary gland is attributed to the scialic acid cotransporter, while in Section 3.4 it is attributed to sodium/iodide symporter (see page 85, lines 29-30).”

RESPONSE: *The PBPK model described in Section 3.4 was developed prior to publication of the study of Qin et al. (2012) in which secretion of nitrate into the salivary gland was attributed to the scialic acid cotransporter.*

Section 3.8 Biomarkers of Exposure and Effect

COMMENT: Charge question: *Are the biomarkers of exposure specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?* Reviewer #3 commented: “The text indicates that there are no biomarkers for exposure to nitrate and nitrite because there are endogenous sources. Although nitrate, nitrite, or metabolites (e.g., urea) could theoretically be measured in blood or urine, and high levels used as an indicator of excessive exposure, in practice they are not. No changes in this section are needed.”

RESPONSE: *No response necessary.*

CHARGE QUESTION: *Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.* Reviewer #3 commented: “The statement that there are no valid biomarkers of exposure is consistent with other sections in the text. Among human studies of potential nitrate/nitrite effects, biomarkers of exposure were not used and Chapter 1 states that tests to measure nitrate or nitrite in blood or urine are not clinically useful.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are the biomarkers of effect specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?* Reviewer #3 commented: “The text of section 3.8.1 correctly notes that the biomarker for effect for hematological effects – methemoglobinemia – is not specific to nitrate/nitrite. Other possible causes of methemoglobinemia are

discussed in this section. There is also a brief mention of detection of N-nitroso compounds in urine as a measure of nitrosation, and the non-specificity of that biomarker is noted. The non-specificity of these endpoints is adequately conveyed and I would not change the text.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are there valid tests to measure the biomarker of effect? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.* Reviewer #3 commented: “Methemoglobinemia is used extensively as a biomarker of effect for nitrate and nitrite as covered in other sections of the text. I found no inconsistencies.”

RESPONSE: *No response necessary.*

Section 3.9 Interactions with Other Chemicals

COMMENT: Charge 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? If not, please clarify and add additional references.* Reviewer #3 commented: “A brief discussion of potential interaction of nitrate with other chemicals on the sodium/iodide symporter in the thyroid, as mentioned elsewhere in the document, should be included here.”

RESPONSE: *The following text was added to Section 3.9: “Nitrate, thiocyanate, and perchlorate are dose-dependent competitive inhibitors of the sodium-iodide symporter (NIS), which mediates the uptake of iodine by the thyroid (DeGroef et al. 2006). Overexposure to any one of these competitive inhibitors could decrease iodine uptake and result in thyroid dysfunction; this effect could be more severe during exposures to combinations of these substances (and possibly other NIS competitive inhibitors).”*

COMMENT: “Nearly all of the interactions covered in this section are with chemicals not likely to be found at hazardous waste sites. This is not an oversight, but a consequence of the existing literature on relevant interactions. I am not aware of any other studies that involve chemicals more commonly found at hazardous waste sites.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? If not, please clarify and provide any appropriate references.* Reviewer #3 commented: “The mechanisms of interaction between nitrite and amino compounds to form nitroso- derivatives are not discussed here; the reader is referred to Section 3.5. Interactions leading to altered severity of forestomach hyperplasia and incidence of forestomach papillomas are summarized without mention of mechanism. It might be useful to clearly state that the mechanism(s) for these apparent interactions is/are unknown if that’s the case. A final paragraph notes two studies in which co-exposure with nitrite decreased a carcinogenic response. Again, no mention is made of potential mechanism.”

RESPONSE: *This section was revised and includes a caveat that mechanisms are not known.*

Section 3.10 Populations that are Unusually Susceptible

COMMENT: Charge question: *Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?* Reviewer #3 commented: “There are populations that have higher susceptibility to nitrate/nitrite toxicity, and I agree with the choices of populations presented. I am not aware of any studies that would lead to including any other populations as unusually susceptible.”

RESPONSE: *No response necessary.*

COMMENT: “There is a discussion of biological factors that make infants more susceptible on page 24. That information should be repeated in this section to make sure that readers interested in infant susceptibility don’t miss it.”

RESPONSE: *A statement was added to Section 3.10 referring the reader to Section 3.7 for detailed discussion of biological factors which may be responsible for increased sensitivity of infants.*

Section 3.11 Methods for Reducing Toxic Effects

General discussion

COMMENT: Charge question: *Is the management and treatment specific for the substance, or is it general for a class of substances?* Reviewer #3 commented: “Some aspects of management are very non-specific, while others are specific to substances that induce methemoglobinemia.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Is there any controversy associated with the treatment? Is it a “well accepted” treatment?* Reviewer #3 commented: “The use of methylene blue to manage methemoglobinemia is well accepted. This sections mentions that hyperbaric oxygen might be of benefit but appropriately notes that its efficacy has not been proven in controlled studies. Methods listed for managing the consequences of methemoglobinemia (e.g., oxygen, benzodiazepines for seizures, etc.) are pretty standard.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?* Reviewer #3 commented: “There are no hazards from treatment unique to populations susceptible to nitrate/nitrite. However, methylene blue treatment is contraindicated in some patients, and this is mentioned in this section. The section also includes alternative approaches for patients in which methylene blue treatment does not work well.”

RESPONSE: *No response necessary.*

Reduced absorption or enhanced elimination

COMMENT: Charge question: *Are treatments available to prevent the specific substance from reaching the target organ(s), or are the actions general for a class of substances?* Reviewer #3 commented: “There are no treatments to prevent nitrate/nitrite specifically from reaching the target organ. As the profile states, activated charcoal can reduce gastrointestinal absorption.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Is there any controversy associated with the treatment? Is it a “well-accepted” treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?* Reviewer #3 commented: “Activated charcoal is well accepted approach to reducing GI absorption of a wide range of substances.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?* Reviewer #3 commented: “There are no unique hazards from activated charcoal administration in populations susceptible to nitrate/nitrite.”

RESPONSE: *No response necessary.*

Preventing adverse effects

COMMENT: Charge question: *Are there treatments to prevent adverse effects as the substance is being eliminated from the major organs/tissues where it has been stored (e.g., as a substance is eliminated from adipose tissue, can we prevent adverse effects from occurring in the target organ[s])?* Reviewer #3 commented: “No such treatments exist.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are treatments available to prevent the specific substance from reaching the target organ(s), or are the treatment’s actions general for a class of substances?* Reviewer #3 commented: “The profile mentions a study suggesting that chlorhexidine mouthwash can diminish conversion of nitrate to nitrite in the mouth. The effectiveness in diminishing toxicity nitrate/nitrite is unknown.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Is there any controversy associated with the treatment? Is it a “well-accepted” treatment? If the discussion concerns and experimental method, do you agree with the conceptual approach of the method?* Reviewer #3 commented: “The use of mouthwash to reduce nitrite formation is not a well-accepted treatment. The conceptual approach is sound, but the efficacy in diminishing toxicity remains to be established. This approach is merely mentioned in the profile, and I don’t think that its potential role in treatment is in any way exaggerated or over-emphasized.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?* Reviewer #3 commented: “There should be no special hazards associated with the use of antibacterial mouthwash in populations susceptible to nitrate/nitrite.”

RESPONSE: *No response necessary.*

Section 3.12 Adequacy of the Database

COMMENT: Charge question: *Do you know of other studies that may fill a data gap? If so, please provide the reference.* Reviewer #3 commented: “I am not aware of any studies that would fill an important data gap.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Are the data needs presented in a neutral, non-judgmental fashion? Please note where the text shows bias.* Reviewer #3 commented: “I did not detect any bias in the presentation of the data needs. They appear objective.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Do you agree with the identified data needs? If not, please explain your response and support your conclusions with appropriate references.* Reviewer #3 commented: “The data needs are clearly justified. I agree with the identified needs.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Does the text indicate whether any information on the data need exists? Reviewer #3 commented: “Each data need is prefaced by a brief summary of the status of existing data on the subject. From these presentations, the articulated data need flows logically.”*

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Does the text adequately justify why further development of the data need would be desirable; or, conversely, justify the “inappropriateness” of developing the data need at present? If not, how can this justification be improved.* Reviewer #3 commented: “The value of filling each data need is usually apparent. Although the case could perhaps be made stronger by following up with a clear statement that “With this information, ... [what could be done or understood that is not now]” this typically has not been done in previous profiles to my knowledge.”

RESPONSE: *Section 3.12 was reviewed to ensure that for each identified data need, statements were made regarding the usefulness of additional information.*

Chapter 4

COMMENT: Charge question: *Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables? Please provide appropriate references for your additions or changes.* Reviewer #3 commented: “I am not aware of any information on chemical or physical properties that is wrong or missing from the tables.”

RESPONSE: *No response necessary.*

Chapter 5

COMMENT: Charge question: *Are you aware of any information that is wrong or missing? If so, please provide copies of the references and indicate where (in the text) the references should be included.* Reviewer #3 commented: “I am not aware of any information that is wrong or missing from this chapter. It would be useful, however, to add some information explaining the presentation of data in Table 5-2. Although the text includes a caution about interpreting TRI data because only certain facilities are required to report, the data in Table 5-2 indicate numbers that look suspiciously like regulatory limits and not actual amounts. This should be explained so that this table is not misinterpreted and its informational value is clearer.”

RESPONSE: *The following text was added to Chapter 5: Facilities that must report to the TRI include industries in a specific business sector such as manufacturing, mining, or electric generation, employ ≥ 10 full-time employees, and manufacture or process 25,000 pounds of a TRI-listed chemical or use $> 10,000$ pounds of a TRI listed chemical per calendar year. Therefore there are some facilities that may be processing or using nitrate and/or nitrite, but are not required to report to TRI because they do not meet the regulatory criteria. The amounts reported in Tables 5-1, 5-2, and 5-3 represent those reported by all facilities in each state that are required to report to the TRI and represent the range of minimum to maximum amounts of each chemical present on site at these facilities during the year.*

Chapter 6

COMMENT: Charge 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.* Reviewer #3 commented: “The information presented adequately covers releases, transport and fate in the environment, and the appearance of nitrate/nitrite in media to which people are exposed. The extent of occurrence is sufficiently display in Figures 6-1 to 6-4 (although a key to the shade coding of the states is needed to understand their meaning). I am not aware of any additional information on these topics.”

RESPONSE: *No response necessary.*

COMMENT: Charge 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.* Reviewer #3 commented: “Information on transport and other aspects of environmental fate in all relevant media is well presented. I am not aware of any relevant information that is missing.”

RESPONSE: *No response necessary.*

COMMENT: Charge 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.* Reviewer #3 commented: “Monitored levels in the environment from relevant sources are presented using proper units. The information is clear regarding the form being measured (nitrate versus nitrite). Discussion of the quality of the information is adequate and I am not aware of any relevant information that is missing.”

RESPONSE: *No response necessary.*

COMMENT: Charge 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?* Reviewer #3 commented: “The significant pathways of exposure for both the general population and workers in occupational settings are presented. Populations with potentially high exposure are clearly identified. I agree with the selection of these populations – they are consistent with current thinking. There are no other populations that should be added in my opinion.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *For Sections 6.8.1, Identification of Data Needs and 6.8.2 Ongoing Studies, answer the same questions presented in Section 3.12.2, Identification of Data Needs and 3.12.3, Ongoing Studies.* Reviewer #3 commented: “This section is well written and clearly identifies where data needs do and do not exist.”

RESPONSE: *No response necessary.*

Chapter 7

COMMENT: Charge question: *Are you aware of additional methods that can be added to the tables? If so, please provide copies of appropriate references.* Reviewer #3 commented: “I am not aware of any additional methods to add to the tables.”

RESPONSE: *No response necessary.*

COMMENT: Charge question: *Have methods been included for measuring Key metabolites mentioned previously in the text?* Reviewer #3 commented: “Ammonia and urea are metabolites of nitrate/nitrite, but are of limited biological significance. Nitric oxide is an important metabolite that has biological effects. However, it is not something that is typically considered as a biomarker for nitrate/nitrite. The text does not contain methods for measuring ammonia, urea, or nitric oxide, but I do not consider this a weakness in this section.”

RESPONSE: *No revisions were suggested. Two analytical methods for determination of ammonia in urine were added to Table 7-1.*

COMMENT: Charge question: *If unique issues related to sampling for the substance exist, have they been adequately addressed in the text? What other discussion should be provided?* Reviewer #3 commented: “When sampling to measure nitrate or nitrite specifically, it is important to prevent any redox interconversion during sample handling which could create artificially high or low values. This issue was not discussed in this section.”

RESPONSE: *Information from Tsikas (2005) was added to the third paragraph in Section 7.1.*

COMMENT: Charge question: *For Section 7.3.1, Identification of Data Needs, answer the same questions presented in Section 3.12.2, Identification of Data Needs.* Reviewer #3 commented: “This section reads like a collection of statement rather than an articulation of data needs regarding analytical methods.”

RESPONSE: *The Exposure portion of Section 7.3.1 was revised to read: “Nitrate and nitrite may be converted to many other compounds in the body, such as N-nitroso compounds, including nitrosamines. Approximately 25% of absorbed nitrate is secreted to saliva and about 20% of this is reduced to nitrite. Nitrite is converted to nitric oxide by the acidic environment on the stomach. Methods exist for the measurement of nitroso compounds and nitrite in plasma and salivary nitrite (Bondonno et al. 2012). Nitrate in the diet may contribute to nitric oxide levels in the body, and increases in these levels can be a biomarker of exposure. Ammonia is a minor urinary product of nitrite/nitrate in which analytical methods are available (Huizenga et al. 1994; Tietz 1970). N-Methylnicotinamide has also been shown to be a potential biomarker of exposure to nitrate/nitrite and there are methods to measure this (Jansen et al. 1995). No data needs were identified.”*

Chapter 8

COMMENT: Charge question: *Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference.* Reviewer #3 commented: “I am not aware of any other regulations or guidelines that would be appropriate to add.”

RESPONSE: *No response necessary.*

Chapter 9

COMMENT: Charge question: *Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional reference.* Reviewer #3 commented: “I do not have additional references to contribute to this section.”

RESPONSE: *No response necessary.*