DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR N-NITROSODI-n-PROPYLAMINE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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Peer reviewers for the third pre-public comment draft of the Toxicological Profile for N-Nitrosodi-n-propylamine were:

Richard J. Bull, Ph.D.
Toxicologist
MoBull Consulting and Washington State University
Richland, WA

F. Peter Guengerich, Ph.D.
Tadashi Inagami Professor of Biochemistry
Department of Biochemistry
Vanderbilt University School of Medicine
Nashville, TN

James Bruckner, Ph.D.
Department of Pharmaceutical and Biomedical Sciences
College of Pharmacy
University of Georgia
Athens, GA

Comments provided by Peer Reviewer #1

General Comments

COMMENT: Chapter 1 adequately summarizes the published health effects literature presented in Chapter 2.

RESPONSE: *No response needed.*

COMMENT: Chapter adequately reflects the published literature on adverse health effects of this chemical. I am not aware of additional studies that might provide information relevant to derivation of MRLs. The text is well organized and clearly written. I made a few minor editorial changes in the text in red ink.

RESPONSE: *The Reviewer's annotated editorial comments were addressed.*

COMMENT: I agree with omission of the acute-duration oral MRL of 0.095 mg/kg/day, due to the absence of pertinent/appropriate data from well-conducted toxicology studies. Terashima et al. (2015) administered the compound daily for 14 consecutive days, rather than giving a single dose, as is typically done in an acute study. These investigators did not examine any other potential target organ, nor assess biochemical or molecular indices of injury in the liver (which may be more sensitive than morphology). The acute MRL of 0.095 mg/kg/day is 950X the EPA (1987) estimate for 1 in 100,000 cancer risk. EPA's calculated 1/10,000, 1/100,000, and 1/1,000,000 cancer risks levels for lifetime exposure to drinking water containing 0.5, 0.05, and 0.005 ug/L of N-nitrosodi-n-propylamine. A copy of the EPA IRIS document is attached.

RESPONSE: *No response needed.*

COMMENT: It would be worthwhile to point out that the EPA has conducted a carcinogenicity assessment for oral exposure to N-nitrosodi-n-propylamine.

RESPONSE: *EPA's cancer weight-of-evidence classification for N-nitrosodi-n-propylamine is presented in Sections 1.2 and 2.19, and in Table 7-1. The oral slope factor of 7.0E+0 (mg/kg/day)$^{-1}$ was added to Table 7-1.*

Specific Comments

COMMENT (page 2, line 27): "National Toxicology Program" should be substituted here for "U.S. Department of Health and Human Services."

RESPONSE: *Regarding the Reviewer's comment in Section 1.2, it is ATSDR's practice to use HHS as the source of the cancer weight of evidence classifications published by NTP; NTP (2016) is listed as the reference.*

COMMENT (page 22, line 35): The values of 25 and 70% pertain to the extent, not the rate of oral absorption.

RESPONSE: *Regarding the Reviewer's comment in Section 3.1.1, "rates" was changed to "extents."*

COMMENT (page 23, lines 6 & 7): It would be useful to provide additional detail on the factors (i.e., site and vehicle) that substantially enhance percutaneous absorption. This information might be useful in avoiding significant dermal exposures.

RESPONSE: *In re-evaluating the dermal absorption data, ATSDR concluded that direct comparisons across the three studies is not possible due to differences in study design. The sentence in question in Section 3.1.1*—"The degree of absorption varied greatly (4–78%) depending on the site of the application and the vehicle used"—*was revised to the following:* "The degree of absorption varied greatly (4–78%); differences in the test animal species, site of the application, and the vehicle used preclude direct comparisons across the studies."

COMMENT (page 23, lines 16-18): It should be stated that N-nitrosodi-n-propylamine was not detected in any organ examined in the other 3 subjects.

RESPONSE: *The text in Section 3.1.2 states that N-nitrosodi-n-propylamine was only detected in the liver of one of the subjects. The following statement*—"N-Nitrosodi-n-propylamine was not detected in the other examined tissues (brain, kidney, and pancreas) from the four subjects"—*was added to indicate that non-detectable levels were found in other tissues.*

COMMENT (page 23, lines 29-34): Can it be concluded from the findings of Lethco et al. (1982) and the other two research groups that nitrosamines are widely distributed in the body. Can any concludsion be reached on concordance of distribution site(s) and tumor target(s)?

RESPONSE: *The text in Section 3.1.2 was revised to note that nitrosamines are widely distributed*— "The limited data available regarding the distribution of related nitrosamines suggest that they are widely distributed in the body."

COMMENT (page 24, line 18): The source of Figure 3-1 should be stated, if it was taken from a published paper.

RESPONSE: *This figure which was included in the 1989 toxicological profile for N-nitrosodi-n-propylamine appears to have been created by ATSDR using information discussed in Section 3.1.3.*

COMMENT (page 26, lines 23-25): Can any interspecies comparisons of carcinogenic potency be made from results of either the oral or subcutaneous cancer bioassays?

RESPONSE: *Given the differences in the study designs (length of exposure, exposure route, and frequency of exposure), differences in the doses tested, and poor survival, it is difficult to make interspecies comparisons of carcinogenic potency from the available data.*

COMMENT (page 26, line 19): Has a PBPK model for any other nitrosamine been published?

RESPONSE: *No PBPK models were identified for N-nitrosodi-n-propylamine. MRLs were not derived for N-nitrosodi-n-propylamine. ATSDR did not conduct a literature search to identify whether PBPK models have been developed for other nitrosamines.*

COMMENT (page 27, lines 17-20): It might be pointed out that ethanol is an inducer of CYP2E1. Thus CYP2E1 inducers other than ethanol could also potentiate the toxicity or carcinogenicity of N-nitrosodi-n-propylamine.

RESPONSE: *The following statement was added to Section 3.2*—"Co-exposure to other compounds that induce CYP2E1 may result in increased metabolism of N-nitrosodi-n-propylamine, which could result in increased toxicity and/or carcinogenicity."

COMMENT (page 46, lines 9-11): Monkeys, of course, are generally accepted as the best animal model for humans. Since CYP2E1 plays a key role in activation of the parent compound, species with hepatic CYP2E1 levels closest to humans should be the most appropriate model. Mice have substantially higher levels than do humans. Rats have lower levels than mice, but somewhat higher activity than humans.

RESPONSE: *The statement referenced by the Reviewer*—"Although toxicity and carcinogenicity of N-nitrosodi-n-propylamine has been demonstrated in rodents and monkeys, the animal species that serves as the best model for extrapolating results to humans may be difficult to identify"—*was deleted from the profile.*

COMMENT (page 36, line 14): I would delete mention of a potential pediatric study.

RESPONSE: *The statement referenced by the Reviewer*—"Studies in young animals and/or children would be useful to address potential concerns of that children may be more susceptible to the toxicity of N-nitrosodi-n-propylamine than adults"—*was revised to:* "Studies in young animals would be useful to address potential concerns that children may be more susceptible to the toxicity of N-nitrosodi-n-propylamine than adults."

COMMENT (page 48, line 3): Have N-nitrosodi-n-propylamine concentrations in fish been adequately measured?

RESPONSE: *ATSDR does not know whether N-nitrosodi-n-propylamne levels have been adequately measured in fish because Section 5.5.4 was not one of the sections updated during the partial update process.*

COMMENT (page 49, line 7): Monitoring the time-course of metabolite excretion would be useful for estimating the rate of metabolism and excretion.

RESPONSE: *ATSDR revised the Absorption, Distribution, Metabolism, and Excretion portion of Section 6.2 to include the following sentence*—"Studies measuring the time-course of metabolite excretion would be useful for estimating the rates of metabolism and excretion."

COMMENT (page 49, line 8): The term “external” or “administered” should be substituted for “exposed”.

RESPONSE: *The discussion of Analytical Methods in Section 6.2 was deleted in an update of the toxicological profile guidance.*

Comments provided by Peer Reviewer #2

General Comments

COMMENT: I had some difficulties with this review. I am not close enough to a University library to retrieve the primary references and most of these papers were old enough that they cannot be retrieved electronically. I could not depend upon the Toxicological profile for design of the key studies (may have Ns for the study identified in one of the graphs, but I could not be sure). This accounts for some of my criticism.

RESPONSE: *No response needed.*

COMMENT: I understand that the intent of the Toxicological Profiles is to provide guidance to people dealing with contaminants of soil, air or water associated with hazardous waste sites. I am concerned that the parameters against which profiles are developed have been fixed to the point it prevents the utilization of informative data to develop guidance in the form of MRLs. This means that no estimate will be made if the data are from studies that do not fall within the strict guidelines for acute, intermediate, and chronic exposures. DNPA belongs to a very toxic group of compounds and is clearly a dangerous compound. I would maintain, based on the limited data available (primarily via the oral route, but with some data on other routes) that is presents an acute, intermediate exposure, and a chronic exposure risk. Therefore, guidance is needed. Instead of providing guidance based on what is known, the document dwells on what is not known related to a very long list of potential effects for where no studies have been performed. Guidance for what is known is better than no guidance at all.

RESPONSE: *Although ATSDR has strict guidelines on what it considers acute, intermediate, or chronic duration exposure, these definitions do not prevent the development of duration-specific MRLs. ATSDR often includes data for other durations (or exposure routes) to support the identification of critical effects and points of departure. As discussed in Appendix A, ATSDR did not consider the available data for this compound to be adequate for MRL derivation.*

In numerous sections of the profile, particularly Sections 1.2 and 2.1, ATSDR summarizes what is known about the toxicity/carcinogenicity of N-nitrosodi-n-propylamine. Regarding the Reviewer's statement "the document dwells on what is not known related to a very long list of potential effects for where no studies have been performed," ATSDR is assuming that the Reviewer is referring to Chapter 2, in which there are "no data" statements for potential health outcomes that have not been assessed. These statements are included in the discussion of health effects so that the reader knows whether these endpoints have been examined and whether effects have been observed.

COMMENT: I am also put off by the way the information is presented. The data that is reviewed is so poorly described that it is impossible to review technically without going back to the original papers (which were not provided and are difficult to retrieve because of their age, i.e. not available electronically). To the extent the data from these studies were discussed it was often confusing, due largely to inclusion of two distinctively unrelated thoughts/information in a single sentence. My recommendation is that the material be presented as simply as the MRLs, themselves, with reference to the source of the data. This could be unraveled by a technical reader if the usual application of UF to NOAELs or LOAELs used by ATSDR to arrive at a safe exposure level are provided. Alternatively, the document could provide sufficient detail for a technical review of the basis of the MRL. The material

provided falls into a mushy spot where there is too much detail for the workers being served and too little for a technical review of the established MRL. Either of the suggested improvements would be better than what is in the document.

RESPONSE: *No MRLs were derived for N-nitrosodi-n-propylamine. It is unclear what section of the profile the Reviewer is referring to; MRLs are presented in Section 1.3 and the MRL Worksheets in Appendix A. Section 1.3 notes whether there are MRLs and presents any values in a table. Detailed discussions of the MRLs are presented in Appendix A; however, this section of the profile is not intended to include a detailed discussion of individual health effect studies. Rather, it is an overview of the route- and duration-specific data with a focus on effects occurring at lower concentrations/doses. Chapter 2 provides a more detailed discussion of health effects and details for individual studies are provided in the Levels of Significant Exposure (LSE) table (Table 2-1). Since no MRLs were derived for N-nitrosodi-n-propylamine, the MRL Worksheets include a discussion of a rationale for why the database was considered inadequate for derivation of an MRL. The rationale for not deriving an acute-duration oral MRL includes brief descriptions of the results of each study.*

COMMENT: Figure 2-1 is confusing in that it only identified the liver as a target organ, but clearly other target organs for cancer were identified in these studies and the graph indicates that histopathology was done. Was there no non-tumor pathology reported? Second, the reference to footnote not identified on the figure. Figure 2-2 is confusing because there is no indication of what the numbers adjacent to the symbols meant. Second, it is not clear what is meant by levels of "significant" exposure. Is this a statistical statement or an indication of which doses resulted in the adverse effects? A clear definition would be helpful. Table 2-1 is a more useful display of the data available. However, the efficacy of "parameters monitored" column is questionable in that measurements of these identified parameters do not appear in the table (numerical or simply positive vs. negative)

RESPONSE: *Regarding the Reviewer's comment on Figure 2-1, the discussion of neoplastic tumors is presented in the cancer effects section, regardless of the tissue. ATSDR notes that the carcinogenicity studies discussed in the profile only reported neoplastic findings. A reference to the footnote was added to the title for Figure 2-1.*

Regarding Figure 2-2, the numbers next to the symbols correspond to the figure keys in Table 2-1; this is defined in the User's Guide in Appendix C. In Table 2-1, the parameters monitored are parameters that were assessed in the study; if a significant alteration in one of these parameters was found, then it is noted in the effects column. The intent of listing the parameters is to give the reader some information on the study design.

COMMENT: Finally, the focus should be on what adverse effects of have been found with DNPA, not a listing of adverse effects that have not been studied. These can be pointed out as deficiencies in the data, but the lack of this data does not prevent derivation of MRLs for the adverse effects that have been documented. It should be sufficient to indicate that none of the studies described were designed to detect a broad range of adverse effects. MRLs can clearly be developed based on these studies. It should be simply stated that there are no data related to other effects that would be of potential interest. This may be used to caveat the derived MRL. EPA goes further by adding an additional UF if such studies have not been conducted. I would prefer a statement that indicates that there simply are no data in other areas of potential concern that simply cannot be addressed. This could be identified as a caution that is associated with limited data.

RESPONSE: *The focus of the discussion of health effects is on adverse health outcomes reported in human and animal studies. ATSDR disagrees with the Reviewer that an MRL can be derived for N-nitrosodi-n-propylamine. The Agency does not believe that the data are adequate to identify the critical target of toxicity (non-neoplastic effects) because the available studies only examined a limited number of potential endpoints. ATSDR has, on occasion, used a modifying factor to account for data gaps. However, this is used for specific gaps; it is not used for the lack of general toxicity studies that are needed to identify the critical targets of toxicity. EPA similarly uses a database uncertainty factor, but this is typically for the lack of adequate developmental toxicity or 2-generation reproduction studies or a specific data gap.*

ATSDR Charge Questions and Responses

QUESTION: Does Chapter 1 adequately reflect the published literature regarding health effects for this substance

COMMENT: In general, yes. I am not aware of other studies that might inform the derivation of an MRL. I do have some editorial comments on this section.

> Endogenous mechanisms for generation of nitrosamines have been recognized for some time (this is not referring to reactions within the stomach, but systemically). In the case of NDMA this formation in humans appears to far exceed exogenous exposures in food, water, and air (Reviewed in Hrudey et al., 2013 Risk Analysis 33(12): 2179). Given an appropriate external or endogenous source of secondary amines, similar formation of other volatile nitrosamines could occur. I am not aware of any studies that document this.
>
> Page 2, line 16. "Carcinogenic Effects" would be grammatically correct.
>
> Page 2, Line 18. I would suggest this sentence should begin: "In rats exposed to DNPA for life, ….. "observed 2 or 5 days a week" is obviously not what was meant.
>
> Page 4, line 7. Too much focus on an overly strict definition of chronic exposure (involving greater than 365 days) excludes a cancer study of 50 weeks duration in mice that would be considered chronic by virtue of the endpoints measured by any reasonable toxicologist. Did this study include any non-cancer pathological or histological examinations that would be useful for developing MRLs (which exclude carcinogenic endpoints)?

RESPONSE: *Regarding the Reviewers editorial comments in Chapter 1 (page 2), "Cancer Effects" was changed to "Carcinogenic Effects" and "(2 or 5 days/week)" was changed to "(administered 2 or 5 days/week)".*

Regarding the comment on the 50-week study, Gruiciute et al. (1982a) did not report whether there were nonneoplastic alterations.

QUESTION: Does Chapter 2 adequately reflect the published literature regarding health effects for this substance? Focus on the current data assessment which resulted in deletion of the acute-duration oral MRL previously derived I the 1969 toxicological profile.

COMMENT: Chapter 2 apparently reflects the limited published literature regarding health effects of DPNA. I know of no other published studies. I see no specific justification for the deletion of the acute-

duration oral MRL in Chapter 2. The rationale for this was provided in the text of the Peer Review Charge under Chapter 2. I find that rationale irresponsible. It essentially indicates that because we have database that only addresses a selection of adverse effects of possible concern, we refuse to provide guidance for the effects we know that DNPA does produce because these other effects could have occurred at a lower dose. The effects shown to result from DNPA treatment are serious effects that should be taken account of even if the literature does not provide data on other hypothetical DNPA-induced health effects of concern. On the other hand, I see no reason to identify a NOAEL or LOAEL related to modifications of a pentobarbital sleep time. The original intent of this parameter was to determine whether a chemical modified the metabolism of pentobarbital at a time when it was difficult to directly demonstrate interference with drug metabolism. If anything, this might indicate that metabolism of DNPA might be also be inhibited at high doses. The significance this finding on toxicity is not clear as such inhibition would be followed by increased synthesis of the affected drug metabolizing enzymes. It is clearly not an adverse effect in the usual meaning of that term.

RESPONSE*: It is ATSDR's practice to not discuss MRLs in Chapter 2 (with the exception of presenting the MRLs in the LSE tables and figures); the rationales for not deriving MRLs for N-nitrosodi-n-propylamine are discussed in Appendix A. As discussed in Appendix A, MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. The available database was not considered sufficient for acute- or intermediate-duration oral MRLs (no chronic duration studies were identified). The available database has only examined body weight and hepatic effects following acute exposure and carcinogenic endpoints following intermediate-duration exposure. Carcinogenicity studies identify several cancer sites, including the liver, gastrointestinal tract (esophagus and forestomach), and respiratory tract (nasal cavity and lungs); it is not known if N-nitrosodi-n-propylamine would also induce non-neoplastic changes in these tissues. Because there is considerable uncertainty that the liver is the most sensitive target following acute-duration oral exposure, an MRL was not derived.*

Regarding the comment on pentobarbital sleep time, the entry for the Nishie et al. (1972) study in Table 2-1 was revised to indicate that the study also found hepatocellular swelling, which is considered a minimally adverse effect.

QUESTION: Do you agree/disagree with the deletion of the acute-duration oral MCL of 0.095 derived in the 1969 toxicological profile? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: I do not agree with the deletion of the acute-duration oral MRL. ATSDR should provide that guidance, whether it is based on the same or another study. There is no need for me to suggest a novel MRL. ATSDR treats these data in a standard way. They simply need to select the study that appears most appropriate and apply standard approaches to arrive at an MRL.

RESPONSE: *As noted in response to previous comments, ATSDR does not consider the available acute-duration database sufficient for derivation of an MRL. The available database has only examined body weight and hepatic effects following acute exposure and carcinogenic endpoints following intermediate-duration exposure. Carcinogenicity studies identify several cancer sites, including the liver, gastrointestinal tract (esophagus and forestomach), and respiratory tract (nasal cavity and lungs); it is not known if N-nitrosodi-n-propylamine would also induce non-neoplastic changes in these tissues. Because there is considerable uncertainty that the liver is the most sensitive target following acute-duration oral exposure, an MRL was not derived.*

QUESTION: Comment on any aspect of the MRL database assessment that you feel should be addressed.

COMMENT: I think that there are enough data to set an intermediate-exposure MRL and chronic-exposure MRLs as well. There are standard techniques that can be applied to project the probability of a carcinogenic effect with lifetime exposures from shorter exposures. In the case of non-cancer endpoints, the chronic doses to induce reversible effects are usually closely approximated by dose that produce a similar incidence of the same effect in a subchronic study. The data are not extensive, but there are real effects that can be anticipated from the available studies. The conclusion that there are no chronic studies because a 50-week study in mice missed the formal definition by 2 weeks is not scientifically defensible nor is it responsible.

RESPONSE: *MRLs are not developed for carcinogenic effects, they are specific for noncarcinogenic health effects. Given that no intermediate-duration studies reported noncarcinogenic effects and no chronic-duration studies were located, MRLs could not be derived for these durations. The 50-week study (Griciute et al. 1982) is not considered suitable for an MRL because the study only reported data for tumor incidences; the investigators did not report whether non-neoplastic lesions occurred.*

QUESTION: Thoughts on the regulations and guidelines that are presented in Chapter 7 and any that should be added or removed. Are you aware of any additional regulations or guidelines that we should add? Are there any that should be removed? Explain.

COMMENT: The California State Water Resources Board, Drinking Water Division has a notification limit (NL) for DNPA of 0.000010 mg/L (available on line). The same NL was set for NDMA and DENA. The derivation of that NL should be in CalEPA's PHG background document (also on line). The NL for carcinogens are generally set at a 10^{-5} added lifetime risk.

RESPONSE: *ATSDR does not typically include state regulations in Chapter 7 of the profile.*

QUESTION: Please address the MRL worksheet (Appendix A) based upon the questions provided above about the MRL.

COMMENT: My comments above are based MRL worksheets presented in Appendix A. The description of the 50-week study in mice indicates that histopathology was done. One would assume that this assessment would have identified non-tumor pathology, but this was not discussed in the document.

RESPONSE: *The investigators for the 50-week study (Griciute et al. 1982) did not discuss any non-neoplastic lesions.*

QUESTION: Please provide comments about the process used in Appendix B.

COMMENT: Appendix B appears to be a standard literature search. Not sure what comments are sought. To evaluate the effectiveness and completeness of the search strategy, I would have to have replicated the search myself.

RESPONSE: *No response needed.*

Comments provided by Peer Reviewer #3:

COMMENT: I agree that Chap. 1 adequately summarizes the published literature regarding the health effects (Chap. 2).

RESPONSE: *No response needed.*

COMMENT: The title compound is not specifically manufactured except for small scale research use. It is not used in any known industrial processes and is found only as a contaminant in relatively trace amounts in the environment.

RESPONSE: *No response needed.*

COMMENT: Essentially all of the studies with the compound have been parts of structure-activity relationships among carcinogenic nitrosamines. In my experience, chemical carcinogenesis of nitrosamines was popular ca. 1980 but today there is relatively little interest in the area, aside from some work with tobacco-specific nitrosamines. Most of the leading investigators in the previous era have retired and/or are deceased.

RESPONSE: *No response needed.*

COMMENT: I agree that the report does adequately reflect the literature regarding health effects of the title compound. I am not aware of other studies with this compound that bear on risk assessment. I agree that the compound should be treated as a probable human carcinogen, based on (i) its general genotoxicity in most assays (*in vitro* and *in vivo*, Tables 2 and 3), (ii) its similarity to the methyl and ethyl homologues, which are clearly carcinogenic in many systems, and (iii) the carcinogenicity observed in multiple rodent studies.

RESPONSE: *No response needed.*

COMMENT: I also agree that an MRL value cannot be estimated from the available data. The studies on carcinogenesis were done with relatively high doses, and even at what appears to be the lowest (oral) dose (1 mg/kg 2 ×/week for 50 weeks) significantly elevated numbers of several tumors were observed (see p. 16).

RESPONSE: *No response needed.*

COMMENT: The *in vitro* genotoxicity data (Table 2-2) do not really allow for extrapolations to humans.

RESPONSE: *No response needed.*

COMMENT: Again, I am not convinced that there is enough data to set an MRL for this genotoxic compound, although I am not convinced that this is a problematic chemical, due to its low occurrence.

RESPONSE: *No response needed.*

COMMENT: I agree with the conclusions and, again, I do not think that an MRL is possible in the absence of more research.

RESPONSE: *No response needed.*

COMMENT: I did have one question about Table 7. The Dept. of Energy (DOE) lists three PAC values (Protective Action Criteria), with a dagger footnote that is not defined. What is the source?

RESPONSE: *The footnote on the PAC values is the letter "f" that is defined at the bottom of the table.*

COMMENT (Section 3.1.3, page 24): Park et al. (1980) did *not* find isopropyl DNA adducts, in contrast to the statement in the text.

RESPONSE: *The profile does not state that Park et al. (1980) found isopropyl DNA adducts; the statement cited to Park et al. 1980 is:* "propylation takes place via a bimolecular reaction*."*

COMMENT (Section 3.1.3, page 25, paragraph 1): I have no explanation for the methylations attributed to *N*-nitroso-n-dipropylamine and question the technology available at the time these papers appeared. There is a finite level of O^6-methylguanine in mammalian DNA due to alkylation by *S*-adenosylmethione and possibly other sources.

RESPONSE: *ATSDR did not identify more recent studies on metabolism.*