Disposition of Peer Review Comments for Toxicological Profile for 1,2-Diphenylhydrazine

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 1,2-Diphenylhydrazine were:

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Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses

Chapter 1

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

COMMENT: Yes, Chapter 1 adequately summarizes the published literature regarding health effects for 1,2-diphenylhydrazine. There are, however, a couple of points on which more clarity would be helpful.

- First, throughout the ATSDR document, the dose is presented in mg/kg for the NCI studies. It is not clear to me how this was calculated from the % feed exposure concentrations in the technical report.
- Second, the hyperkeratosis/acanthosis in the stomach is presented as a non-neoplastic lesion for male and female rats at the high dose (15 mg/kg). Although the effect is most prominent in the high dose male rat, it is also present in the low dose female rat (table with data from technical report below). Therefore, I suggest either limiting discussion to male rat (if those are the only incidences that are high enough to warrant consideration) or including this finding at the low dose with both male and female rats.

	Control low	Control high	Low	High
Male rat – hyper.	0	2	1	10
Male rat – acanth.	0	2	4	17
Female rat – hyper	0	0	3	2
Female rat – acanth.	0	2	6	5

- Finally, the liver was the only tissue assessed in the Dodd study because it was identified as an important target tissue in the cancer studies. I got the impression from the text that the liver was identified as an important tissue in the Dodd study, but that is not the case, as the Dodd study was not a complete subchronic toxicity evaluation.

RESPONSE: *The mg/kg/day doses for the NCI (1978) study were calculated using the reported time-weighted average (TWA) diet concentrations (0.008 and 0.03% for males and 0.004 and 0.01% for females) and EPA reference body weights (0.380 and 0.229 kg for males and females, respectively) and food intakes (0.030 and 0.021 kg/day for males and females, respectively).*

The incidence of hyperkeratosis was only statistically different from control groups in the high-dose male rats. The text in Section 2.6 was revised to reflect this information: "Statistically increased incidences of hyperkeratosis and acanthosis in the stomach occurred in male rats at 24 mg/kg/day and acanthosis was observed in female rats at 3.7 mg/kg/day 1,2-diphenylhydrazine in the diet for 78 weeks (NCI 1978)."

The Dodd et al. (2012) study focused on potential liver effects and would not be considered a "complete subchronic study." Support for identifying the liver as the most sensitive target comes from the NCI (1978) study, which examined a wide-range of potential endpoints.

Chapter 2

QUESTION: Does Chapter 2 adequately reflect the published literature regarding health effects for this substance?

COMMENT: Yes, Chapter 2 adequately reflects the published literature regarding health effects for this substance. There were some discrepancies between the narrative and Table 2-1, which I hope the authors can address (noted in the document). Related to this, it would be helpful to get more clarity and consistency on when biological changes rise to the level of adverse. For example, the narrative includes discussion of minor liver weight increases and hepatic enzyme changes, but these do not seem to be reflected in the NOAELs in Table 2-1. I assume this is because they were not considered to be adverse, but this was not explicitly stated. Also, it is not clear if there are specific criteria for determining when a change rises to the level of adverse (e.g., what level of increased liver weight would be considered adverse?). If ATSDR has guidance on that or uses guidance from other agencies, that would be good to reference.

RESPONSE: *ATSDR does not consider changes in organ weight, in the absence of histological alterations, to be biologically relevant effects. Table 2-1 was revised to indicate the observed changes in serum enzyme levels observed in the Dodd et al. (2012) studies were not considered biologically relevant.*

- *5-day study:* "Slight decrease (13%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no alterations in hepatic serum enzymes or liver histopathology"
- *2-week study:* "Slight decrease (12%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no other alterations in hepatic serum enzymes or liver histopathology"
- *4-week study:* "13% and 26% reductions in serum alkaline phosphatase and aspartate aminotransferase, respectively, at 15.5 mg/kg/day were not considered biologically relevant; no alterations in liver histopathology"

QUESTION: Intermediate-Duration Oral MRL: A newly developed intermediate-duration oral MRL of 0.05 mg/kg/day was derived based on a NOAEL of 4.80 mg/kg/day for hepatic effects in rats exposed to 1,2-diphenylhydrazaine in the diet for 13 weeks (Dodd et al. 2012). This NOAEL was divided by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

Is the principal study cited for this derivation properly interpreted?

COMMENT: Yes, the Dodd study is properly interpreted.

RESPONSE: *No response necessary.*

QUESTION: Are the uncertainty factors appropriate and are they applied correctly?

COMMENT: Yes, the uncertainty factors are appropriate and applied correctly.

RESPONSE: *No response necessary.*

QUESTION: Do you agree that the derivation of the new intermediate-duration oral MRL of 0.05 mg/kg/day is valid? Explain.

COMMENT: Yes, I agree that the derivation of the new intermediate-duration oral MRL of 0.05 mg/kg/day is valid. First, the Dodd et al. 2012 study appears to be well designed and conducted, and clearly reported, making it a good study for MRL derivation. I agree with the rationale for using the NOAEL instead of a benchmark dose calculation due to the extremely steep dose-response data. The selection of 4.8 mg/kg/day as the basis of the MRL is further strengthened by the consistency of findings across multiple non-neoplastic lesions in the liver. Furthermore, I agree with the application of two uncertainty factors to the NOAEL based on animal to human extrapolation and accounting for human variability.

RESPONSE: *No response necessary.*

QUESTION: Is the final value of the MRL acceptable?

COMMENT: Yes, the final value of the MRL is acceptable.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included that may be relevant in the derivation of other inhalation or oral MRLs for this chemical?

COMMENT: No, I am not aware of any additional studies.

RESPONSE: *No response necessary.*

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

COMMENT: The emphasis in the section on rationale for not deriving a chronic oral MRL felt a bit off target. My assumption is that because there was not a NOAEL for the non-neoplastic lesions in the NCI study and because there was a "recovery" period, a chronic MRL might not actually be protective. I did not think the lack of a dose-response was a strong argument, since 0/1, 4, and 10 for acanthosis and 0/4, 12, 16 for lung inflammation in the male rat, seem like reasonably good dose response relationships to me. Furthermore, there is consistency between the male and female in that both exhibited the same types of lesions, albeit with a lack of dose-response in the female rats. In short, I could have gone either way on developing a chronic oral MRL. Therefore, if the argument could be strengthened as to why it was not conducted, that would be helpful. Some possibilities: 1) if a chronic MRL would not be more conservative than the intermediate MRL (because the dose dose that would provide the basis would be similar) then it does not add value to provide it. 2) Since you concluded that the evidence for the non-neoplastic respiratory/gastro endpoints is "not classifiable" and that is presumably the data that would be used to develop the chronic oral MRL, is this an argument for not developing a chronic MRL?

RESPONSE: *The worksheet for the chronic-duration oral MRL in Appendix A was revised and the lack of dose-response data is no longer listed in the rationale for not deriving an MRL:* "The only available chronic-duration oral study was not considered suitable for derivation of an MRL due to the long duration (28–30 weeks) between exposure termination and histological examination and methodological problems with the only available study."

Chapter 5

QUESTION: We would like you to review text mainly for citations more recent than 1990. Those primarily address uses and approval for use.

COMMENT: No problems identified with citations after 1990. Very minor grammatical fixes noted in Chapter 5.

RESPONSE: *No response necessary.*

Chapter 7

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

COMMENT: No, I am not aware of additional regulations or guidelines relevant to 1,2-diphenylhydrazine.

RESPONSE: *No response necessary.*

QUESTION: Are there any that should be removed? Explain.

COMMENT: No, I did not think any that you have included should be removed.

RESPONSE: *No response necessary.*

Appendix A

QUESTION: Please address the MRL worksheet based upon the questions provided above about the newly derived MRL.

COMMENT: Overall, I found the worksheet to be presented clearly and really appreciated the tables included in Appendix A.

RESPONSE: *No response necessary.*

Appendix B

QUESTION: Please provide comments about the process utilized in this section.

COMMENT: The process presented for the literature search appears sound and thorough.

RESPONSE: *No response necessary.*

Appendix C

QUESTION: Please provide comments about the systematic literature review process presented in this section.

COMMENT: It is not clear from the description exactly how the abstract and full-text screening was conducted. In effect, details were not provided on the conduct of the systematic literature review. Were any software programs used for data extraction? How many people screened the abstracts, full text, or evaluated risk of bias? Was there a requirement for more than one screener to assess the identified literature at any of the phases? Did screeners undergo any kind of training or validation process to make sure findings were relatively consistent? These are details that I feel are usually included in systematic review descriptions.

I appreciated the step-by-step description of rating the confidence in the body of evidence, translating to a level of evidence, and making hazard identification conclusions. These sections were very clear and the tables, along with figure C-1, provided transparency in the process.

RESPONSE: *ATSDR did not use software programs for data extraction; the abstracts and full studies were evaluated by an experienced toxicologist. The principal author of the profile screened the literature search, evaluated the abstracts and full studies, completed the data extraction, and evaluated the risk of basis. The literature search (with abstracts), full studies, detailed summaries of the toxicological studies, and risk of bias analysis were made available to the ATSDR Chemical Manager for review.*

Annotated Comments

The Reviewer suggested a number of editorial revisions, most of the suggested revisions were made to the profile. Some stylistic changes that were purely arbitrary were not incorporated. Responses to Reviewer comments that were not considered editorial or stylistic are presented below.

COMMENT: Mention that exposure might result from occupational exposure or pharmaceutical use?

RESPONSE: *Section 1.1 was revised to include occupational exposure. Both pharmaceutical products are no longer available for human use. Below is the revised text:*

> "The general population is not likely to be exposed to 1,2-diphenylhydrazine in the environment; exposure may also occur in workers involved in the manufacture or use of 1,2-diphenylhydrazine."

COMMENT: Since this is the same study that is referenced in the first sentence of the paragraph, I suggest changing to "In the chronic study".

RESPONSE: *The suggested revision was made in Section 1.2.*

COMMENT: Left Justify tissue labels in Figure 1-2.

RESPONSE: *The suggested revision was made to Figure 1.2.*

COMMENT: What about decrease in ALP at 15.5?

RESPONSE: *Regarding the comment on the Dodd et al. (2012) 5-day study in Table 2-1, the following revision to the text in the Effect column was made:* "Slight decrease (13%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no alterations in hepatic serum enzymes or liver histopathology."

COMMENT: Same question as above

RESPONSE: *Regarding the comment on the Dodd et al. (2012) 2-week study in Table 2-1, the following revision to the text in the Effect column was made:* "Slight decrease (12%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no alterations in hepatic serum enzymes or liver histopathology."

COMMENT: This seems very comparable to the reduction seen at the earlier time points (5-day and 2-week), but it was not noted for those entries. Also confusing that it is listed here, but the NOAEL does not reflect it being considered as adverse. Maybe note here that the decreases in ALT and AST (which was significantly decreased at 10.3) were not considered to be adverse. Whatever is decided, I suggest making it consistent across the time points in terms of including or not including.

RESPONSE: *Regarding the comment on the Dodd et al. (2012) 4-week study in Table 2-1, the following revision to the text in the Effect column was made:* "13% and 26% reductions in serum alkaline phosphatase and aspartate aminotransferase, respectively, at 15.5 mg/kg/day were not considered biologically relevant; no alterations in liver histopathology."

COMMENT: There were incidences of 6 in the 3.7 and 5 in the 9.2 – I am not sure that a difference of 1 incidence is meaningful to conclude that it was happening at one dose but not the other. Is this strictly based on your statistical evaluation of the data?

RESPONSE: *Regarding the comment on the NCI (1978) 78-week rat study in Table 2-1, the incidence of acanthosis of the stomach was not statistically significant at 9.2 mg/kg/day, as compared to the high-dose control group. ATSDR conducted the statistical analyses of the NCI (1978) incidence data.*

COMMENT: I looked through the NCI report and could not find where the doses were calculated that are used in this report. If food consumption was not reported – how were the doses calculated? Were they estimated based on the Dodd paper? I think this should be included somewhere and referenced before we get all the way to describing effects.

RESPONSE: *Regarding the comment in Section 2.3, doses were estimated using the reported TWA diet concentrations (0.008 and 0.03% for males and 0.004 and 0.01% for females) and reference body weights (0.380 and 0.229 kg for males and females) and food intakes (0.030 and 0.021 kg/day.*

COMMENT: This did not make it into table 2-1 – is it because the effect was considered to not be toxicologically significant? Are there a priori cut-offs for what rises to the level of inclusion in the table? So far, I thought it was just based on statistical significance, which would include this endpoint and change the NOAEL in the table.

RESPONSE: *Regarding the comment in Section 2.9 on the increased relative liver weight reported in the Dodd et al. (2012) study, this slight increase in liver weight was not considered biologically relevant for the liver and not included in the Table 2-1. In the absence of histological alterations, ATSDR does not consider alterations in organ weight to be adverse.*

COMMENT: This is inconsistent with Table 2-1. I am not sure why the decrease in ALP would be noted in the table for the 4 week, but not for the 5-day and 2-week.

RESPONSE: *Regarding the comment in Section 2.9 on the decreases in serum alkaline phosphatase reported in the Dodd et al. (2012) study, Table 2-1 was revised to include the decreases in alkaline phosphatase levels in rats exposed for 5 days or 2 weeks:*

- *5-day study:* "Slight decrease (13%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no alterations in hepatic serum enzymes or liver histopathology"
- *2-week study:* "Slight decrease (12%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no other alterations in hepatic serum enzymes or liver histopathology"

COMMENT: Not included in entry for the 4 week study in table 2-1.

RESPONSE: *Regarding the decrease in aspartate aminotransferase reported in the Dodd et al. (2012) 13-week study, Table 2-1 was revised to include these alterations. The following is the revised Effect text:* "13% and 26% reductions in serum alkaline phosphatase and aspartate aminotransferase, respectively, at 15.5 mg/kg/day were not considered biologically relevant; no alterations in liver histopathology."

COMMENT: Could another word be used here? Not sure about dominated in this context. Maybe dictated? Or influenced?

RESPONSE: *Regarding the comment in Section 5.1, the following change was made:* "The fate, transport, and distribution of 1,2-diphenylhydrazine in the environment are influenced by its rapid oxidation to azobenzene."

COMMENT: Not sure what is meant by "on standing".

RESPONSE: *The referenced sentence in Section 5.2.4 was revised to delete the phrase "on standing":* **"**Results of treatment by wet air oxidation are in keeping with the observation that 1,2-diphenylhydrazine oxidizes to azobenzene (Riggin and Howard 1979)."

COMMENT: Check hyperlink. I was redirected.

RESPONSE: *Regarding the hyperlink for NTP (2016) in Table 7-1, the hyperlink was corrected.*

COMMENT: In the description of the Dodd study above, both the exposure concentration and calculated dose were presented. In this section, it is not clear how the doses presented were derived from

the exposure concentrations in the feed. For example, were those estimated by ATSDR or presented in the NCI technical report.

RESPONSE: *Regarding the comment in the chronic-duration oral MRL worksheet in Appendix A, ATSDR only includes dose calculations for the MRL principal study. A full study summary was not included for the NCI study because an MRL was not derived for chronic oral exposure. The doses for the NCI study were calculated by ATSDR.*

COMMENT: Looks like there were stomach lesions in the 6.3 mg/kg dose group, they just weren't statistically significant.

RESPONSE: *The incidence of acanthosis of the stomach in the male rats exposed to 6.3 mg/kg/day was not significantly different from the control incidence.*

COMMENT: True for female rats, but looks like there is a dose-response for male rats.

RESPONSE: *ATSDR agrees that there is an apparent dose-response relationship for stomach acanthosis in the male rats.*

COMMENT: In some places in the body of the report, hyperkeratosis is listed along with acanthoses. Should this be added to the table?

RESPONSE: *Hyperkeratosis was not added to Table A-3 because it did not occur at the low dose in the male or female rats. The NOAEL and LOAEL values for hyperkeratosis in rats was added to Table A-2:*

Table A-2. Summary of Relevant NOAEL and LOAEL Values in Rats and Mice Following Chronic-Duration Oral Exposure to 1,2-Diphenylhydrazine[a]

	Males		Females	
	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)
Fischer 344 rats				
Increased mortality				9.2
Decreased body weight gain	6.	24	3.7	9.2
Interstitial lung inflammation		6.3		3.7[b]
Acanthosis of stomach	6.3	24		3.7[b]
Hyperkeratosis of stomach	6.3	24	9.2	
Fatty metamorphosis in liver	6.3	24	3.7	9.2[c]

Table A-2. Summary of Relevant NOAEL and LOAEL Values in Rats and Mice Following Chronic-Duration Oral Exposure to 1,2-Diphenylhydrazine[a]

	Males		Females	
	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)
B6C3F1 mice				
Increased mortality		69		69
Decreased body weight	14	69	6.9	69
Coagulative hepatic necrosis			6.9	69

[a]Rats and mice were exposed for 78 weeks followed by a 28–30-week observation period.
[b]This effect was not observed in rats exposed to 9.2 mg/kg/day.
[c]Incidence higher than low-dose control group, but not high-dose control group.

Source: NCI 1978

COMMENT: This answers my previous question about statistics. However, it is pretty buried in the appendix. If it could be mentioned when the data is discussed, that would be helpful.

RESPONSE: *Regarding the Reviewer's comment on the footnote in Table A-3 indicating statistical significance of lesion incidence, Chapter 2 is a synthesis and evaluation of the weight of evidence for health endpoints rather than a detailed discussion of the specific studies. Health outcomes discussed in the text and presented in the LSE tables and figures typically have incidences or values that are significantly different from controls.*

COMMENT: 2.19 and 2.20 are cancer and genetox results, so I think they should be included here.

RESPONSE: *Regarding the Reviewer's comment on the following sentence in Section C.3* "A summary of the extracted data for each study is presented in the Supplemental Document for 1,2-Diphenyl-hydrazine and overviews of the results of the oral and dermal exposure studies (no inhalation exposure studies were identified) are presented in Sections 2.2–2.18 of the profile and oral data are summarized in the Levels Significant Exposures table in Section 2.1 of the profile (Table 2-1)." *Sections 2.19 (Cancer) and 2.20 (Genotoxicity) were not included in the list because cancer and genotoxic effects are not included in ATSDR's systematic review.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

Front Matter

COMMENT: Charge to reviewers indicates that *"[t]his toxicological profile is an update of a previous profile on this substance published in 1990. It also includes text from an Addendum published in 2009."* My own literature research revealed publication of *"Toxicological Profile for Hydrazines"* (ATSDR 1997). Version history therefore should clarify that 1,2-diphenylhydrazine, the subject substance, is not included among hydrazines addressed in the 1997 document, just as the 1997 document explicates that *"The term "hydrazines" is a generic name used in this document to describe a group of three structurally related chemicals: hydrazine, 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine… Numerous other hydrazine derivatives exist as well. For example, the reader is referred to the Toxicological Profile for 1,2-Diphenylhydrazine (ATSDR 1990) for information on this chemical."*

RESPONSE: *ATSDR disagrees with the Reviewer that a statement should be added to the Version History that 1,2-diphenylhydrazine is not included in the hydrazines toxicological profile. The Toxicological Profile for Hydrazines clearly states that 1,2-diphenylhydrazine is discussed in a separate profile. The Version History section is intended to include a history of toxicological profiles and addendum that discuss the subject substance.*

COMMENT: In my opinion, the title of the Toxicological Profile, and I believe of all of ATSDR's Tox-Profiles, is awkward and possibly even incorrect: *Toxicological Profile for 1,2-Diphenylhydrazine.* In my view, toxicological profiles are not "for" substances. They are *for* people; they are profiles "of" substances. I suggest adopting a generic Tox-Profile title that is grammatically correct, such as (in the present case): *Toxicological Profile of 1,2-Diphenylhydrazine.*

RESPONSE: *ATSDR will consider the Reviewer's suggestion of a title change in future versions of the toxicological profile guidance.*

Chapter 1

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

COMMENT: Chapter 2, not Chapter 1, summarizes the published literature regarding health effects of 1,2-diphenyhydrazine, whereas Chapter 1 succinctly summarizes Chapter 2. The Chapter 1 summarization is cursory but, in the context of the detailed information presented in Chapter 2, it is informative and appropriately succinct. It begins ineloquently, however, with the vague statement that *"Information on the toxicity of 1,2-diphenylhydrazene is limited."* Such vagueness may be appropriate in advertising ("seats are limited," not infinite), but the statement amounts to an inappropriate truism for a scientific report, even for a summary section. The statement indeed is applicable to any substance whatsoever, whether the amount of information available on the substance is 'a little' or 'a lot', as long as it is not infinite which, of course, it cannot be. I imagine that the statement was intended to mean that 'little' information rather than 'a lot of' information was available (whatever these amounts imply), and/or that available information was found to be inadequate to implement at least some purposes of the Toxicological Profile.

The statement should be clarified, briefly but unambiguously, for this summary chapter, reflecting the subsequent content of Chapter 1 and the rest of the Tox-Profile.

RESPONSE: *The first sentence in Section 1.2, which stated that toxicity information was limited, was revised:*

> "Information on the toxicity of 1,2-diphenylhydrazene is derived from a small number of health effect studies."

COMMENT: Effects observed only in animals are likely to be of concern to humans. The Tox-Profile appropriately states that such concern is *"presumed"* with respect to hepatic effects. When effects are observed *"only in animals,"* the presumption of concern is appropriate when the meaning of the phrase is that the effects were not *looked for* in humans. That is the case with respect to 1,2-diphenylhydrazine. The presumption of concern would be less appropriate if the effects indeed were looked for in people, but were not found, despite having been looked for.

RESPONSE: *No response needed.*

COMMENT: The form of the exposure (and/or dose) parameter "mg/kg/d" used in Chapter 1 and throughout the Tox-Profile, and commonly used in ATSDR Tox-Profiles, is mathematically ambiguous. A correct, unambiguous expression of this term is "mg/(kg d)." This notation would distinguish the term from the unintended "(mg d)/kg)," which also could be derived from "mg/kg/d" [via mg x 1/(kg/d) = mg x d/kg = (mg d)/kg)]. That is, day (d) belongs in the denominator, whereas algebraic manipulation of the expression "mg/kg/d" can place it in the numerator or in the denominator. This ambiguous notation, being easy to correct, should be corrected.

RESPONSE: *ATSDR notes that the term "mg/kg/day" is widely used in the literature, but will consider using an alternate term in future revisions of the toxicological profile guidance.*

Chapter 2

QUESTION: *Does Chapter 2 adequately reflect the published literature regarding health effects for this substance?*

COMMENT: Text indicates that *"[t]he primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 1,2-diphenylhydrazine."* As an overall perspective, Chapter 2 reflects adequately the findings in published literature. Indeed, the coverage seems thorough and clear, and its graphical presentation is creative and communicative. As explained below, however, it excludes *unpublished* data evinced from inquiries of pharmaceutical and dye industry users of 1,2-diphenyl-hydrazine, if such inquiries never were made.

The Tox-Profile states that *"No epidemiology or human exposure studies are available, and data are restricted to a few oral studies in laboratory animals"* (page 1). The scope of animal studies reviewed in the Tox-Profile is narrow. It includes a small number of appropriate and adequately designed studies involving standard bioassay species (most notably rodents). The literature search on which it was based, however, spans an appropriately broad range of health endpoints, including cancer and non-cancer endpoints.

I examined my archive of data, and found no published literature that would add to the inventory or elucidation of already-included 1,2-diphenylhydrazine health effects potentially posed to humans or to animals. I also conducted a Google search consisting of descriptors *"1,2-diphenylhydrazine AND toxicology."* It produced 15,500 hits. Presumably, this number would be increased with reasonably expanded scope of descriptors, including terms such as health effects, health risks, clinical effects, and so forth.

I also conducted a second Google search, using the descriptors *"1,2-diphenylhydrazine AND MSDS OR "Safety Data Sheet."* It produced 15,800 hits. These numerous Google hits represent a potential information resource excluded from the 1,2-diphenylhydrazine Tox-Profile. MSDSs (Material Safety Data Sheets) or nowadays, SDSs, typically do not undergo *external* peer review, as might be arranged by the editor of an academic journal. They typically do undergo *internal* peer-review. Internal peer review may be at least as rigorous as external peer review, given corporate liability issues that must be addressed via completeness and accuracy of disclosure of subject-substance characteristics in a published (or, in lawsuits, a discoverable) SDS or MSDS prepared with respect to a commercial product that must be handled safely by consumers.

RESPONSE: *ATSDR conducted an extensive literature search to identify health effect data published after the 1990 toxicological profile. Health effects data discussed in toxicological profiles are based on the primary literature. ATSDR also uses the grey literature (e.g., unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations) and secondary sources (e.g., review articles) to identify papers not identified from other sources. With few exceptions, ATSDR does not cite health effects data to secondary sources. ATSDR considers MSDSs and SDSs to be secondary or tertiary sources of health effects data. These documents do not always provide citations for health data and there is therefore no way to determine if the information in these reports is derived from studies already cited in the profile.*

QUESTION: We would like you to focus on the current data assessment which resulted in the derivation of the new MRL. **Intermediate Duration Oral MRL:** A newly developed intermediate-duration oral MRL of 0.05 mg/kg/day was derived based on a NOAEL of 4.80 mg/kg/day for hepatic effects in rats exposed to 1,2-diphenylhydrazaine in the diet for 13 weeks (Dodd et al. 2012). This NOAEL was divided by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability). Is the principal study cited for this derivation properly interpreted?

COMMENT: The principal study (Dodd, *et al.* 2012) supporting derivation of the intermediate-duration oral MRL of 0.05 mg/(kg d) is interpreted properly. The study is described in detail in Appendix A of the Tox-Profile. This study was selected over two other studies (Marhold, *et al.* 1968; NCI 1978), both of which focused on possible 1,2-diphenylhydrazine carcinogenicity. I have no problem with the *reasoning* presented for selection of the Dodd, *et al.* (2012) rat study, and it might be fortuitous because the study is the most recent, and it is modern.

One relevant selection issue was omitted, however, and should be addressed. Specifically, postdating the 1968 and 1978 studies, the system of classifying liver lesions in rats was replaced based upon continuing research (Maronpot, *et al.* 1986). This raises the issue of whether (and to what degree) results obtained in rat studies utilizing the older liver lesion classification system are comparable to results utilizing the more modern classification system. The present Tox-Profile does cite Maronpot (1983 and 1986), but only with respect to lung tumors.

RESPONSE: *A note was added to Section 2.19 to indicate that the term neoplastic nodule is no longer recommended by NTP: "*ATSDR notes that the nomenclature for classifying proliferative hepatocellular

lesions was revised and the term "neoplastic nodule" is no longer recommended by the National Toxicology Program (NTP) to describe lesions that would now be termed hepatocellular hyperplasia or hepatocellular adenoma (Maronpot et al. 1986a)." *The use of the now obsolete term neoplastic nodule does not appear to change the interpretation of the data that the liver is a target of 1,2-diphenylhydrazine carcinogenicity given that increases in the incidence of hepatocellular carcinomas were also reported in rats and mice tested in the NCI (1978) study.*

QUESTION: Are the uncertainty factors appropriate and are they applied correctly?

COMMENT: "Uncertainty factors" were applied correctly in deriving the intermediate-duration oral MRL in the Tox-Profile, including a factor of 10 for extrapolation from animals to humans and another factor of 10 for human variability, totaling UF = 100. This leaves open the issue of whether a *safety factor*, or *safety factors*, also should be applied. The answer may be 'no', but in that case, the formalism *"uncertainty factor"* should be changed to *"safety and uncertainty factors,"* thereby clarifying that both parameters together amounted to 100, and both are encompassed in the derived MRL. A formal distinction between the concepts of safety factors and uncertainty factors should be added to the text, including discussion of the precautionary principle and its proper place, if any, in ATSDR Tox-Profiles. This applies, of course, throughout the subject Tox-Profile and, I would say, to all ATSDR Tox-Profiles.

RESPONSE: *ATSDR does not use safety factors for deriving MRLs. The Agency uses defined uncertainty factors to account for possible pharmacokinetic and pharmacodynamic differences between humans and experimental animal species, to protect sensitive human subpopulations, and when the threshold for toxicity is poorly defined (i.e., a NOAEL value has not been identified). This is same approach that EPA uses to derive reference doses/concentrations. The U.S. Food and Drug Administration (FDA) uses a safety factor approach to calculate the maximum acceptable daily intake; these factors account for similar uncertainties inherent in extrapolating information from experimental animal toxicity studies.*

QUESTION: Do you agree that the derivation of the new intermediate-duration oral MRL of 0.05 mg/kg/day is valid? Explain.

COMMENT: I am inclined to believe that use of the Dodd, *et al.* (2012) study produced a valid oral intermediate-duration MRL. Pathological changes in rat livers that would have been classified as malignant under the older rat liver lesion classification system might be relegated to non-malignant pathology in the newer system, possibly with *increased* potency with regard to non-cancer effects. I here illustrate the potential significance of the issue. Specifically, in the same year as the NCI (1978) study [and postdating the Marhold, *et al.* (1968) study], another study was conducted that formed the basis for quantifying the cancer potency of chlorinated dioxins (2, 3, 7, 8-TCDD) primarily based upon liver lesions induced in a two-year bioassay using rats (Kociba, *et al.* 1978). Under the liver lesion classification system then in place, the resulting U.S. EPA potency estimate was the highest ever obtained. My own role in this (in the 1980s) was as Chairperson of the *Science Advisory Panel* of the State of Maine, which was addressing dioxin issues related to the paper industry. The paper industry called attention to the issue of liver lesion classification, resulting in my requesting the Dow Chemical Company to release the Kociba, *et al.* (1978) study pathology slides for re-evaluation. Based upon the resulting re-evaluation, the new liver lesion classification system significantly reduced the incidence of rat liver neoplastic response to 2, 3, 7, 8-TCDD exposure, but the issue is complex, and worth addressing in support of MRL derivation in the Tox-Profile.

RESPONSE: *As noted in the response to a previous comment on the re-classification of "neoplastic nodules," ATSDR has added text to Section 2.19 to discuss this issue. Without re-examination of the liver histology slides from the NCI (1978) study, it is not known if the increase in neoplastic nodules would be classified as hepatocellular hyperplasia or carcinoma. However, this would not have influenced the derivation of the intermediate-duration MRL since the neoplastic nodules were reported in a chronic-duration study. As discussed in Appendix A, ATSDR did not consider the NCI (1978) study to be suitable for derivation of a chronic-duration MRL due to the long duration between exposure termination and histological examination and methodological problems with the NCI (1978) study.*

QUESTION: If you disagree, please propose an appropriate intermediate-duration oral MRL and give your rationale.

COMMENT: I do not disagree.

RESPONSE: *No response needed.*

QUESTION: Is the final value of the MRL acceptable?

COMMENT: The MRL appears to be acceptable. It would appear to be even more acceptable if issues raised in this peer review document ultimately are addressed. These most notably include more strongly supporting selection of the Dodd, *et al.* (2012) study for MRL derivation, and addressing the possible value of qualitative and/or quantitative structure-activity relationships (SARs; discussed below, later) in informing MRL derivation.

RESPONSE: *Three studies evaluated the toxicity of 1,2-diphenylhydrazine following intermediate-duration oral exposure, and the Dodd et al. (2012) study was considered the strongest basis for the MRL. The following text is included in Appendix A:* "Due to incomplete details of study design and lack of histopathology data in the 4-week dose-finding study (NCI 1978) and the Marhold et al. (1968) study, derivation of the provisional MRL for hepatic effects is based on findings in the multi-dose study by Dodd et al. (2012). The selected study provides the best available data for characterizing the dose-response relationship for liver effects in laboratory animals orally exposed to 1,2-diphenylhydrazine for intermediate durations and it identified the lowest reliable LOAEL value." *In general, use of SAR data in toxicological profiles is limited and it is not clear how data on a structurally-related compound would increase the confidence in the MRL.*

QUESTION: Are you aware of any studies that are not included that may be relevant in the derivation of other inhalation or oral MRLs for this chemical?

COMMENT: As indicated earlier, I found no published literature that would add to the inventory or elucidation of 1,2-diphenylhydrazine health effects potentially posed to humans (or to animals) reported in the draft Toxicological Profile under review. I do suggest two additional sources of such information. The Tox-Profile reports that "*1,2-diphenylhydrazine* [was] *previously used as an intermediate in dye manufacturing (e.g., benzidine) and an intermediate in some pharmaceuticals.*" The preparer therefore should identify past pharmaceutical and industrial users of 1,2-dphenylhydrazine, and (as suggested earlier, above) contact them to request information about studies that they may have conducted to elucidate its toxicology, and develop safety precautions needed to protect against its potential risks, for use in their operations, or that otherwise may have emerged from their experience.

RESPONSE: *Included in the literature search is a search of EPA's Toxic Substances Control Act Test Submissions (TSCATS) database, which includes industry-sponsored studies submitted to EPA. Identified studies are included in the toxicological profiles; however, no unpublished studies were identified for 1,2-diphenylhydrazine. Unpublished studies submitted by industry to ATSDR are evaluated for inclusion in the profile; however, it is not ATSDR's practice to solicit unpublished studies from industry.*

COMMENT: Appendix A, page 3 of the Tox-Profile indicates that *"[t]here are insufficient data for derivation of an acute-duration inhalation MRL."* Appendix A, page 6 indicates that *"[t]here are insufficient data for derivation of an acute-duration oral MRL."* As noted above, however, numerous MSDSs are available, which appear to constitute a potential information source excluded from consideration in developing the Tox-Profile. A strength of MSDSs generally (in my own experience) is their tendency to focus on acute toxic effects, consistent with short-term exposure of consumers or applicators to freshly opened products. Thus, I suggest exploring the MSDS database to see what, if any, data might be presented regarding short-term exposure, including lethal and non-lethal poisoning. Perhaps such data might enable derivation of an acute-duration oral MRL, and/or an acute-duration inhalation MRL based upon occupational studies.

RESPONSE: *As noted in the Response to a previous comment, ATSDR does not consider MSDSs or SDSs to be reliable sources of toxicity data. In response to the Reviewer's comment, ATSDR did review several publicly available MSDS/SDS for 1,2-diphenylhydrazine and did not locate any acute toxicity data that could be considered for MRL derivation.*

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

COMMENT: The *Forward* indicates that *"[t]his profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed."* This statement, interpreted literally, would include externally peer-reviewed studies published in academic literature, as well as internally peer-reviewed studies (more commonly undertaken in corporate settings), which might not appear in the published literature. ATSDR's Toxicological Profiles might serve the public need better if the more inclusive criterion of *credibility* is applied to both internally and externally peer reviewed studies, whether or not they have been published. This would open the door to asking for proprietary (if also credible) information from industries, as is done routinely in documents prepared under the Toxic Substances Control Act (TSCA). Such requests often produce filings of TSCA notifications to the U.S. Environmental Protection Agency, which must respect filers' possible requests for their confidentiality.

RESPONSE: *ATSDR considers available unpublished studies in evaluating health effects and derivation of MRLs. ATSDR uses EPA's TSCATS database as a source of unpublished studies.*

Chapter 5

QUESTION: We would like you to review text mainly for citations more recent than 1990. Those primarily address uses and approval for use.

COMMENT: Only a few citations in Chapter 5 postdate 1990 (TRI16 2017; US EPA 2005; and US FDA 2016). Potentially valuable updated use information is reported, such as: 1,2-diphenylhydrazine is used in the pharmaceutical industry for producing phenylbutazone (trade name Butazolidin, an anti-inflammatory agent) and sulfinpyrazone (trade name Anturane, a uricosuric agent for the treatment of

gouty arthritis)… but *"phenylbutazone is no longer marketed for human use in the United States, but is still listed for veterinary use (FDA 2016)."*

RESPONSE: *Only a couple of sections in Chapter 5 were updated, these include information on National Priorities List (NPL) sites containing 1,2-diphenylhydrazine (Section 5.1), uses (Section 5.2.3), and Toxics Release Inventory (TRI) release data (Section 5.3).*

Chapter 7

QUESTION: We would like to know your thoughts on the regulations and guidelines that are presented and any that should be added or removed: Are you aware of any additional regulations or guidelines that we should add? Please provide citations. Are there any that should be removed? Explain.

COMMENT: Chapter 7 addresses succinctly and appropriately the issue of applicable international and national regulations, advisories, and guidelines. The text cautions that the list is not exhaustive; it also should report the search cut-off date(s) for inclusion of regulations, advisories, and guidelines. The cautionary statement suggests the possibility that guidelines and/or regulations of great importance to Tox-Profile users might have been omitted. The chapter appropriately presents numerous guidelines and regulations, with the most recent promulgated (by UN IARC) in 2017. The information presented seems reasonably thorough, up to the search cut-off date(s). One concern inherent in a non-exhaustive list of guidelines, advisories, and regulations is possible exclusion of the most stringent. The text provides no assurance that the most stringent are included. This issue should be addressed. I agree that the list need not be "exhaustive." An encyclopedic presentation that might be of value in a litigation setting might be dense and impenetrable for most users of ATSDR Toxicological Profiles. The text also cautions, as it should, that *"current regulations should be verified by the appropriate regulatory agency."*

RESPONSE: *Information on the literature search dates are included in Appendix B. It is not possible to assess what guidelines/regulations would be highly relevant to a particular user. The purpose of the statement in Chapter 7 that the list is not exhaustive is to inform the reader that other guidelines/ regulations may be available. Table 7-1 includes a list of guidelines/regulations that ATSDR typically includes in a profile; the table also states whether there are no values for a particular guideline/ regulation. The Agency does not attempt to identify the most stringent guidelines/regulations; but rather includes national guidelines/regulations that are most frequently used by its users.*

Appendix A

QUESTION: Please address the MRL worksheet based upon the questions provided above about the newly derived MRL.

COMMENT: Appendix A documents and explains how ATSDR derived its intermediate-duration oral MRL for 1,2-diphenylhydrazine. This discussion is encyclopedic. This level of detail is appropriate for the Appendix, conveniently providing in-document technical information for users requiring it. The encyclopedic coverage supports MRL derivation appropriately presented more generally in the main body of the Tox-Profile. My comments on specific MRLs, or their absence, are provided in text above, and will not be repeated here.

RESPONSE: *No response is needed.*

COMMENT: The decision to derive no MRL for acute-duration oral exposure and for chronic-duration oral exposure, and no MRLs at all for inhalation exposure, based upon data insufficiency seems premature. In the circumstance of data insufficiency, I recommend seeking literature on structurally similar substances, in the present case other hydrazine congeners such as 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine. No indication that this was attempted is included in the Tox-Profile, even though it cites a study of structure-activity relationships (SARs), in Section 5.4.2 (page 39) on 1,2-diphenyl-hydrazine *Transformation and Degradation* in air.

Application of available strategies such as analysis of SARs may generate only inadequate data for derivation of toxicity values. This possibility is irrelevant. The important criterion of due diligence is doing the diligent thing, which is to try untried strategies if feasible, such as examining SARs, including qualitative and quantitative SARs.

I am unaware of specific studies that are not included in the profile that may be relevant to deriving MRLs for any of the 1,2-diphenylhydrazine congeners. Even so, the Profile will support adequately the decision to derive, or not derive, MRL values only after feasible possibilities for doing so have been exhausted. In short, reasonable strategies should be followed, and the resulting decision explicated. My concern is that neither has been done, or done adequately.

RESPONSE: *It is not currently ATSDR's practice to base MRLs on structurally related compounds.*

Appendix B

QUESTION: Please provide comments about the process utilized in this section.

COMMENT: Appendix B presents ATSDR's protocol for completing the literature search and screening for the health effects chapter of the Tox-Profile. The discussion is deficient in three respects:

--1. it is general, failing to describe separately the literature search strategy (or strategies) applied to each specific topic area,

--2. it is non-specific, omitting the cut-off date of the literature search generally, and with respect to the search in each topic area [for example, as highlighted above regarding the cut-off date(s) for searching for regulations, advisories, and guidelines], and

--3. it is incomplete, omitting specific health effect topics, as well as topics outside of the health effect area (see below).

Appendix B fails to encompass the SARs issue highlighted earlier. The literature search nonetheless may encompass adequately the broad scope of health effects literature specifically related to 1,2-diphenyl-hydrazine. Specific areas, some exemplified in earlier comments, suggest that the breadth of description in Appendix B might be overly non-specific regarding some issues. As noted earlier, I found no discussion of the search strategy for international and national guidelines and regulations.

As a general comment, information search strategies should be described in more detail in connection with each specific (reasonably delineated) topic area, rather than merely broadly, to assure completeness of toxicological issue coverage. The cut-off date for literature searches should be specified. Appendix B Table B-2 is useful but still inadequate in this regard. Table B-2, for example, reports access dates for PubMed (2017.03), Toxline (2017.03), and Toxcenter (2017.03). These dates are not tied to any specific

topic area, and do not preclude other searches. Further, the query strings seem to be defined *a priori*, but that is not how searches typically are conducted. My own searches, for example, usually depend upon what I encounter, which is to say, they are not defined, or completely defined, *a priori*.

RESPONSE: *ATSDR typically conducts one literature search to identify all relevant data for the profile. It clearly states in Section B.1.1 that the literature search was conducted in March 2017 and that the literature search was intended to update the existing toxicological profile and was restricted to January 1988 to March 2017. ATSDR disagrees with the Reviewer that the literature search strategy employed by ATSDR is not typical of how literature searches are conducted. ATSDR notes that other government agencies, such as EPA, use a similar approach to conducting literature searches. If necessary, ATSDR will conduct additional literature searches on very specific topics, these additional searches would also be documented in Appendix B. For 1,2-diphenylhydrazaine, additional searches were not conducted. Since ATSDR does not typically discuss structure-activity relationships, special searches on this topic were not included.*

Appendix C

QUESTION: Please provide comments about the systematic literature review process presented in this section.

COMMENT: The purpose of Appendix C is to present "ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to 1,2-diphenylhydrazine" (page C-1). In this endeavor, Appendix C is highly successful. Two strengths deserve highlighting: --1. it is generic, and --2. the generic framework is filled in with Tox-Profile-specific data (for example, in Table C-3).

An important caveat regarding Appendix C relates to carcinogenicity. Figure 1-1 (in Chapter 1) depicts the finding of liver cancer in rats exposed chronically to 4 mg/(kg d). Regarding carcinogenicity, however, Appendix C indicates that "ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including DHHS, EPA, and IARC" (page C-10). This statement should requires justification, without which it suggests failure to undertake an analysis within ATSDR's expertise, and within its purview for preparing Tox-Profiles."

RESPONSE: *ATSDR disagrees with the Reviewer that the referenced statement requires justification. ATSDR does not establish weight-of-evidence classifications for carcinogenicity in the toxicological profile, and thus, carcinogenicity data were not subject to a systematic review to establish hazard identification conclusions.*

Comments provided by Peer Reviewer #3:

ATSDR Charge Questions and Responses

Chapter 1

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

COMMENT: Yes

RESPONSE: *No response necessary.*

Chapter 2

QUESTION: Does Chapter 2 adequately reflect the published literature regarding health effects for this substance?

COMMENT: Yes

RESPONSE: *No response necessary.*

QUESTION: Intermediate-Duration Oral MRL: A newly developed intermediate-duration oral MRL of 0.05 mg/kg/day was derived based on a NOAEL of 4.80 mg/kg/day for hepatic effects in rats exposed to 1,2-diphenylhydrazine in the diet for 13 weeks (Dodd et al. 2012). This NOAEL was divided by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

Is the principal study cited for this derivation properly interpreted?

COMMENT: Yes

RESPONSE: *No response necessary.*

QUESTION: Are the uncertainty factors appropriate and are they applied correctly?

COMMENT: Yes

RESPONSE: *No response necessary.*

QUESTION: Do you agree that the derivation of the new intermediate-duration oral MRL of 0.05 mg/kg/day is valid? Explain.

COMMENT: Yes, I agree, because this is the best information and the best study in the literature about this compound, so the MRL is well supported by the selected data.

RESPONSE: *No response necessary.*

QUESTION: Is the final value of the MRL acceptable?

COMMENT: Yes, it is acceptable.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included that may be relevant in the derivation of other inhalation or oral MRLs for this chemical?

COMMENT: No

RESPONSE: *No response necessary.*

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

COMMENT: Nothing to comment.

RESPONSE: *No response necessary.*

Chapter 5

QUESTION: We would like you to review text mainly for citations more recent than 1990. Those primarily address uses and approval for use.

COMMENT: I am not aware of other uses of this compound.

RESPONSE: *No response necessary.*

Chapter 7

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

COMMENT: I found more information on this topic. Please see comments in the main document.

RESPONSE: *No response necessary.*

QUESTION: Are there any that should be removed? Explain.

COMMENT: No, there is nothing to remove in terms of references of regulations or guidelines.

RESPONSE: *No response necessary.*

Appendix A

QUESTION: Please address the MRL worksheet based upon the questions provided above about the newly derived MRL.

COMMENT: The derivation of the MRLs is well explained in a clear way and also the info is organized in several worksheets in a user friendly and straightforward way. Please see comments in the main document to improve Table A-2.

RESPONSE: *No response necessary.*

Appendix B

QUESTION: Please provide comments about the process utilized in this section.

COMMENT: The literature search and inclusion criteria are presented in a detailed and transparent form. Particularly liked figure B-1.

RESPONSE: *No response necessary.*

Appendix C

QUESTION: Please provide comments about the systematic literature review process presented in this section.

COMMENT: I liked very how the framework was organized and the criteria used to extract the key studies. Tables and the integration of data were also informative and well organized.

RESPONSE: *No response necessary.*

Annotated Comments

The Reviewer suggested a number of editorial revisions, most of the suggested revisions were made to the profile. Some stylistic changes that were purely arbitrary were not incorporated. Responses to Reviewer comments that were not considered editorial or stylistic are presented below.

COMMENT: Do not agree that this is a NOAEL because No LOAEL was observed. It could be greater than that value >15.5.

RESPONSE: *Regarding the comment on the NOAEL of 15.5 mg/kg/day for body weight effects identified in the Dodd et al. (2012) 5-day study reported in Table 2-1, 15.5 mg/kg/day was the highest dose tested and was considered the study NOAEL for this endpoint.*

COMMENT: Do not agree that this is a NOAEL because No LOAEL was determined

RESPONSE: *Regarding the comment on the NOAEL of 15.5 mg/kg/day for hepatic effects identified in the Dodd et al. (2012) 5-day study reported in Table 2-1, 15.5 mg/kg/day was the highest dose tested and was considered the study NOAEL for this endpoint.*

COMMENT: The Reviewer made the following comment on the NOAEL of 15.5 mg/kg/day for body weight and hepatic effects for the Dodd et al. 2012 2-week study reported in Table 2-1: **"**Do not agree that this is a NOAEL because No LOAEL was determined"

RESPONSE: *As noted in the previous responses to this comment, 15.5 mg/kg/day was the highest dose tested and was considered a NOAEL for these endpoints.*

COMMENT: Is it possible to include more information on the exposure?

RESPONSE: *Regarding the comment on the Marhold et al. 1968 study in the Exposure Parameter column of Table 2-1, the exposure parameter column is intended to include information on the exposure duration and subroute. There is no additional information to include in this column. ATSDR notes that the Marhold et al. (1968) paper provides limited information on the lethality study and does include information on the doses tested.*

COMMENT: Do not agree that this is a NOAEL because No LOAEL was observed. It could be noted as greater than that value > 15.5.

RESPONSE: *Regarding the comment on the NOAEL of 15.5 mg/kg/day for body weight effects identified in the Dodd et al. (2012) 4-week study reported in Table 2-1, 15.5 mg/kg/day was the highest dose tested and was considered the study NOAEL for this endpoint.*

COMMENT: Do not agree that this is a NOAEL because No LOAEL determined. Is 12.79% considered an adverse effect? It seems not, so maybe it would be better to say that.

RESPONSE: *Regarding the comment on the NOAEL of 15.5 mg/kg/day for hepatic effects identified in the Dodd et al. (2012) 4-week study reported in Table 2-1, 15.5 mg/kg/day was the highest dose tested and was considered the study NOAEL for this endpoint. The 12.79% decrease in alkaline phosphatase levels was not considered biologically relevant. The Effect text was revised to indicate this determination:* "13% and 26% reductions in serum alkaline phosphatase and aspartate aminotransferase, respectively, at 15.5 mg/kg/day were not considered biologically relevant; no alterations in liver histopathology."

COMMENT: The doses tested should be added. Again no LOAEL is determined so do not agree that this is a NOAEL.

RESPONSE: *Regarding the comment on the NOAEL of 19 mg/kg/day for body weight effects identified in the Marhold et al. (1968) 288-day study reported in Table 2-1, as noted previously, the highest dose tested is considered a NOAEL if no significant alterations were observed. Dose information was added to the table.*

COMMENT: Please see comment 10

RESPONSE: *Regarding the comment on the serious LOAEL of 54 mg/kg/day for death identified in the NCI (1978) 4-week rat study reported in Table 2-1, please see the response to the next comment.*

COMMENT: Not clear how this NOAEL was determined, because it is death and the serious LOAEL is defined as 54 and what about the females? What happened with the other doses?

RESPONSE: *Regarding the comment on the NOAEL of 2,600 mg/kg/day for body weight effects identified in the NCI (1978) 4-week rat study reported in Table 2-1, ATSDR identifies NOAEL and LOAEL values for each endpoint, independent of effects which may occur in other tissues. NCI (1978) only reported lethality and body weight effects for the 4-week study. Increases in mortality were observed at ≥54 mg/kg/day and no consistent alterations in body weight were observed.*

COMMENT: Again I cant follow this value with the LOAEL of 390. Also the other 390 is for Females?

RESPONSE: *Regarding the comment on the NOAEL of 6,700 mg/kg/day for body weight effects identified in the NCI (1978) 4-week mouse study reported in Table 2-1, as noted in the response to the previous comments, NOAEL and LOAEL values are identified for each endpoint.*

Regarding the comment on whether the 390 mg/kg/day is a serious LOAEL for gastrointestinal effects in female mice, the table was revised to indicate that the serious LOAEL is 391 mg/kg/day for male mice and 950 mg/kg/day for female mice.

COMMENT: See comment 11.

RESPONSE: *See the response to the previous comment regarding the gastrointestinal effects.*

COMMENT: Figures 2.2 are very difficult to follow although there are explanations at the appendix D on how to interpret them.

RESPONSE: *No response necessary.*

COMMENT: If possible, complete with the number of deaths/total animals treated.

RESPONSE: *Regarding the number of animals that died in the 4-week NCI study, Table 2-1 was revised to include the number of rats (*"2/5 males died at 54 mg/kg/day; 100% mortality at higher doses") *and mice (*"1/5 males and 4/5 females died") *that died.*

COMMENT: This phrase should be removed. There is no however, and there is no more sensitive test strain in this context. The mutagenicity in this case is yes or no.

RESPONSE: *Regarding the deletion of the following sentence:* "However, the mutagenesis studies in *S. typhimurium* were only weakly positive (Dunkel et al. 1985) or positive in a single, more sensitive test strain (TA97)", *the suggested revision was made.*

COMMENT: Is it possible to state the duration of exposure?

RESPONSE: *Regarding the following sentence in Section 2.20:* "In *in vivo* studies (Table 2-3), 1,2-diphenylhydrazine inhibited testicular DNA synthesis in mice when administered as a single 100 mg/kg intraperitoneal injection (Seiler et al. 1977)…," *the sentence notes that the mice received a single dose of 1,2-diphenylhydrazine.*

COMMENT: Bullets should be removed and linked to the first paragraph

RESPONSE*: Regarding Section 3.1, the bulleted text is consistent with ATSDR guidelines for this section of the profile. The intent is to give the reader a quick overview of the available toxicokinetic data.*

COMMENT: There is an estimated value of 1.12 – 1.18 g/cm3, please see EPA dashboard https://comptox.epa.gov/dashboard/dsstoxdb/results?utf8=%E2%9C%93&search=1%2C2-Diphenylhydrazine.

RESPONSE: *As noted in the Version History and Section 1.1 of the profile, this update of the toxicological profile for 1,2-diphenylhydrazine focused on health effects information; the density parameter in the physical-chemical properties table (Table 4-2) was not reviewed in this revision.*

COMMENT: There is a measured value of water solubility of 1.20 x 10E-3 mol/L which corresponds to 221 mg/L please see chemistry EPA dashboard https://comptox.epa.gov/dashboard/dsstoxdb/results?utf8=%E2%9C%93&search=1%2C2-Diphenylhydrazine.

RESPONSE: *This partial update of the toxicological profile was focused on health effects information; the water solubility parameter in the physical-chemical properties table (Table 4-2) was not reviewed in this revision.*

COMMENT: Write the full name, titles of figures and tables should be auto-explicative.

RESPONSE: *Regarding the title of Figure 5-1, this is consistent with ATSDR guidance. Note that the phrase NPL is defined in the paragraph preceding the figure.*

COMMENT: Change types of markers in the legends, it is hard to differentiate the regions. Maybe use different shades of grey, or colors.

RESPONSE: *ATSDR uses the hash marks in Figure 5-1 to facilitate converting the final profile into a Section 508 compliant pdf file. Section 508 of the Rehabilitation Act of 1973 requires that Federal agencies make their electronic information accessible to people with disabilities.*

COMMENT: This information is out of the context here below the figure. Please remove because it is already stated in the beginning of the chapter.

RESPONSE: *The statement* "There is a total of 26 unique sites out of 1,854 hazardous waste sites" *at the bottom of Figure 5-1 was deleted.*

COMMENT: The bullets should be removed and the text written in a single paragraph.

RESPONSE: *Regarding Section 5.1, the bulleted text is consistent with ATSDR guidelines for this section of the profile.*

COMMENT: What about anturane? Is it still in use?

RESPONSE: *Anturane is no longer in use in the United States. The following statement was added to Section 5.2.3:* "Sulfinpyrazone has been withdrawn for sale in the United States (FDA 2009)."

COMMENT: Don't think this is needed.

RESPONSE: *ATSDR has deleted the referenced sentence in Section 5.2.4:* "Wet air oxidation can effectively treat aqueous waste streams that are too dilute to incinerate, yet too toxic to treat using biological processes."

COMMENT: In this case it would be interesting to explain what would be the route of exposure. Also it seems that it would more likely in this case that the population would be exposed to the degradation product as well, depending on the time of residence of the compound in the sites. I would separate this phrase from the other two statements (sulfinpyrazone therapy and occupational exposure in the manufacture of the product.

RESPONSE: *Regarding the statement in Section 5.7*—those living near hazardous waste sites where 1,2-diphenylhydrazine is present—*there are insufficient data to assess the potential exposure routes for individuals living near hazardous waste sites containing 1,2-diphenylhydrazine.*

COMMENT: EPA Dashboard indicates some specific regulations for specific uses of water. A drinking water standard was derived by Nebraska Drinking Water with a value of 0.36 ug/L but I was not able to find the information of how this value was calculated. Please see https://actorws.epa.gov/actorws/toxval/v01/toxval_source?source=actor&casrn=122-66-7.

RESPONSE: *Regarding the Reviewer's comment on Table 7-1, ATSDR does not typically include state guidelines/regulations in the toxicological profile.*

COMMENT: Remove mg/Kg/day of all the values of the table and insert in the head of the table (below Noael and Loael)

RESPONSE: *The suggested revision to Table A-2 was made.*