DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR DISULFOTON

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

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

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NOTE: Peer reviewer comments are written next to "COMMENTS:" in unformatted text. Any italicized text following the comment is added for clarification purposes. Any page and line numbers that were added by the Reviewers have been kept, but often will not align with the appropriate text.

Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: I agree with the reported effects of disulfoton in humans as reported. I am not aware of any additional adverse health effects of disulfoton.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: The toxicity of disulfoton, its mechanism of action and its metabolism are very similar if not at times identical in animals and in humans. Hence, all effects seen in animals are of relevance for humans.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Where potential exposure during disulfoton manufacture is discussed (section 1.1) should it be indicated that this is "hypothetical" given that disulfoton is banned in the USA and in the EU?

RESPONSE: *The reviewer was commenting on text in section 1.1 (Overview and U.S. Exposures) which reads ""People who manufacture, handle, or apply disulfoton or who are involved in the disposal of disulfoton are at a higher risk of exposure than the general population."*

The sentence was edited to match previous statements in the section stating the disulfoton pesticide were canceled by the EPA. The sentence highlighted by the reviewer was edited to read as follows:

> *"People who manufacture, handle, or apply disulfoton or who are involved in the disposal of disulfoton are at a higher risk of exposure than the general population, though occupational exposure is not expected to occur in the U.S. since its cancellation."*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Yes, they do reflect the published literature. Adding a summary statement at the end of each sub-section would be useful.

RESPONSE: *Per ATSDR's Guidance for the preparation of toxicological profiles: "Where possible, use a topic sentence at the beginning of the paragraph." The first sentence of a paragraph summarizes the resulting discussion of health effects when appropriate. The critical endpoints of neurological and developmental effects are summarized in section 1.2 (Summary of Health Effects).*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: There are only limited human studies involving disulfoton. Mostly are case reports. The existing studies have been identified, they have been well described in terms of health effects and exposure. This latter issue is rightly indicated as the most critical, as human exposure levels are mostly unknown.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Yes, for the most part all these variables have been considered. Please see also comments on Appendix C. At times, as indicated in a following section, description and interpretation of some animal studies could have been better.

RESPONSE: *The reviewer's only comment regarding Appendix C is the following: "This is an excellent Appendix that provides a lot of important and useful information, as it is an in-depth analysis of studies on disulfoton. This analysis provides the framework for establishing a level of confidence for each study based on the evaluation of several important and established parameters."*

No revisions were suggested.

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Most if not all studies with disulfoton were carried out in rodents (rats and mice). These are appropriate species for testing disulfoton as the mechanism of action and the metabolism of this insecticide is the same in animals and humans.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, dose-response considerations were an integral part of the analyses contained in this Toxicological Profile for Disulfoton

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No, I am not aware of any other study.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: No, I am not aware of any other study.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: All appropriate NOAELs or LOAELS seem to have been identified, according to the reviewer's knowledge (original studies have not been reviewed by the reviewer).

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: The document makes a good case for distinguishing between serious and less serious effects to be associated with the LOAEL. By reading the details in Tables 2.1, 2.2, and 2.3, it is apparent that this is a correct and useful distinction.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: A relevant issue not discussed in detail in this section relates to the studies which have investigated the development of tolerance to disulfoton and its underlying mechanisms. These aspects

were described in the 1995 ATSDR "Toxicological Profile of Disulfoton" from which much information present in the current document were derived, but have not been included in the present document. The additions that are being proposed pertain to Section 2.15 (Neurological Effects) after oral administration, specifically on pp. 77-78. It is stated correctly that upon repeated exposures to disulfoton in rats or mice, animals become resistant to its toxicity, despite profound inhibition of acetylcholinesterase activity. A reference that could be added here is the following review on this topic (Costa LG et al. Toxicology 1982, attached). The mechanism of such tolerance was identified by several studies in changes (decreases) in muscarinic and nicotinic receptors in the organism (brain, ileum, pancreas, lymphocytes), to compensate for the excessive cholinergic stimulation by accumulating acetylcholine. The most relevant papers in this regard are cited, but the overall "message" is not clearly presented, as most comments relate to the degree of acetylcholinesterase activity. Please note that in some cases papers are listed in the References but do not seem to have been cited in the text. In this regard, please note that a study by McDonald et al. 1988 (listed in References, but not cited or discussed in the text) attempted identifying potential behavioral consequences of such decreases in muscarinic receptors in brain, specifically in the hippocampus. A deficit in spatial memory was found in animals that were tolerant to disulfoton and had low levels of acetylcholinesterase activity and reduced muscarinic receptor density.

RESPONSE: *Additional text and explanation was added to Chapter 2 to in response to this comment The reviewer provided and recommended the Costa et al. (1982) study which was also added into Chapter 2. Specifically, the following text was added to section 2.15 (Neurological) replacing the text that is shown in strikethrough:*

> *"Intermediate-duration studies in animals indicate tolerance to disulfoton is developed over time. Clinical signs of cholinergic toxicity appear initially and diminish while cholinesterase activity remains inhibited, which is characteristically observed in organophosphate pesticides (Costa et al. 1982).~~"In intermediate-duration studies, typical signs of cholinergic poisoning are generally seen only during the first few days, after which they diminish. However, cholinesterase activity usually remains inhibited during exposure."~~*

In addition, the underlined text was also added in Section 2.15 (neurological).

> *"However, with subsequent dosing, the severity of the overt cholinergic effects diminished, while cholinesterase remains inhibited, <u>indicating a tolerance to disulfoton developed</u>."*

Additionally, the McDonald et al. (1988) study was taken out of the reference list and not included in the discussion of neurological health effects in Chapter 2, as the study only exposed animals to intraperitoneal doses. Additionally, the reviewer noted that several studies including the McDonald et al. (1988) were included in the Chapter 8 reference list but not discussed in the text. Each reference was cross-checked to the Profile text and any that were not cited were removed from inclusion in the reference list.

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: The conclusions appear appropriate.

RESPONSE: *No revisions were suggested.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: All these aspects have been well described. There is a good and informative graph on p. 95 on disulfoton metabolism.

RESPONSE: *No revisions were suggested.*

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

COMMENT: No PBPK or PBPD models for disulfoton have been identified.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: Yes, there is a good comparative discussion of disulfoton metabolism between rodents and humans which is very informative and underlines the similitudes.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: Young individuals are considered more susceptible to the acute toxicity of many organophosphorus insecticides; however, only limited information is available for disulfoton. Also, there is concern about potential developmental neurotoxicity of organophosphorus insecticides, but there is no specific information available in case of disulfoton.

RESPONSE: *No revisions were suggested..*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: Genetic polymorphisms in certain metabolizing enzymes may render an individual more susceptible to the toxicity of an organophosphorus insecticide. In case of disulfoton, this may pertain to one of more cytochromes P450. However, this has not been investigated.

RESPONSE: *No revisions were suggested..*

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

COMMENT: Most disulfoton metabolites detected in the urine are not specific for disulfoton but could result from exposure to other diethyl organophosphorus insecticides. The presence of disulfoton sulfoxide or disulfoton sulfone in the urine would be specific for disulfoton, but only one study provided some qualitative data in humans.

The section on p. 104 (lines 1875-1886) related to the measurements of catecholamines and metabolites as markers of exposure to disulfoton is not very useful, as measurements are not specific for disulfoton, and may be associated with many other conditions or exposures.

RESPONSE: *Section 3.3.1 (Biomarkers of Exposure) discusses that catecholamines and metabolites can be used as biomarkers of exposure for disulfoton and discusses that these biomarkers are not specific to disulfoton. This information is useful as these biomarkers have been used in the literature to determine disulfoton exposure, however additional context was added to clarify that these biomarkers are not specific to disulfoton and can be indicative of exposure to other organophosphates*

In Section 3.3.1 (Biomarkers of Exposure), detail was added regarding disulfoton sulfoxide and sulfone and acknowledging the lack of data, as follows:

> *"An unknown amount of disulfoton sulfoxide and/or demeton S-sulfone was detected in the urine from a person exposed to an unknown amount of disulfoton (Yashiki et al. 1990). Disulfoton sulfoxide and disulfoton sulfone are specific to disulfoton but are only reported in this one study."*

In the same section, further detail was added to explain these biomarkers are not specific to organophosphates:

> *"Because organophosphates and carbamates are known to cause an accumulation of acetylcholine at the nerve synapses leading to acetylcholinesterase inhibition (King and Aaron 2015), and since the secretion of catecholamines are influenced by acetylcholine (Norman and Henry 2015), it is likely that other organophosphates can also cause a release of catecholamines from the adrenals and the nervous system."*

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

COMMENT: As for other organophosphorus insecticides, inhibition of red blood cell acetylcholinesterase remains the best biomarker of effects for disulfoton. It is not specific for disulfoton but it has been shown that it reflects inhibition of the same enzyme activity in target tissues. The fact that lymphocyte acetylcholinesterase activity dose not correlate as well with target tissues is also well described. Are there any information on disulfoton and plasma cholinesterase (pseudocholinesterase)?

RESPONSE: *In toxicological studies described in Chapter 2 of the Profile, plasma cholinesterase typically precedes inhibition of red blood cell acetylcholinesterase activity in the brain; however, plasma cholinesterase recovers more quickly than red blood cell acetylcholinesterase following exposure. In Section 3.3.2 Biomarkers of Effect, additional information and references were added to explain that plasma cholinesterase (butyrylcholinesterase) has been used as a biomarker of effect in conjunction with acetylcholinesterase and clinical symptoms of toxicity. The following was added to Section 3.3.2:*

> *"Plasma cholinesterase (also known as serum cholinesterase, pseudocholinesterase, or butyrylcholinesterase) is used to support acetylcholinestase and clinical manifestations to diagnose organophosphate toxicity (Moon and Chun 2014; Strelitz et al. 2014; Worek et al.*

2005). Experimental studies of animals exposed to disulfoton has observed that plasma cholinesterase activity reduces more rapidly than acetylcholinesterase but recovers quicker (Klaus 2005a, 2005b; Sheets 2002). In one clinical case, a man who accidentally ingested disulfoton had depressed serum cholinesterase activity up to 8 days after exposure and cholinergic signs of toxicity (Yashiki et al. 1990). A relationship between plasma cholinesterase and clinical symptoms of organophosphate poisoning has been observed (Mahadeshwara et al. 2013; Work et al. 2005) and plasma cholinesterase appears to be most accurate for acute prognosis of organophosphate poisoning (Aygun et al. 2002). However, use of plasma cholinesterase on its own as a diagnosis tool is not agreed upon (Berger et al. 2005) since it is most commonly used as a biomarker of liver function (Zivkovic et al. 2014)."

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: The section on interactions focuses on the effects of inducers and inhibitors of cytochromes P450 and describes the possible scenarios of relevance for disulfoton.

RESPONSE: *No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: The interactions between disulfoton, the organophosphate chlorpyrifos and the carbamate propoxur is correctly described (p. 108, lines 1997-2002).

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: Nothing to add

RESPONSE: *No revisions were suggested.*

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

COMMENT: Nothing to add

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: It is stated that disulfoton has been banned in the USA in 2009, and all existing quantities were sold by 2011. Could it be indicated here why registration of disulfoton was terminated by the EPA? It is not clear (or not known) whether disulfoton is still produced/sold/used in other countries and if it could find its way into the United States. The fact that disulfoton was not detected in food in recent years (2017-2019) suggest that it does not find its way into the US food supply. Additionally, even when disulfoton was approved in the United States dietary exposure levels were well below the Reference Dose.

RESPONSE: *In Chapter 5, the following detail was added as to why it was terminated:*

> *"Disulfoton is not permitted for use as a pesticide in the United States as of 2009 following voluntary cancellation orders from a few companies that produce disulfoton-containing pesticides."*

Journals, reviews, and government and organizational databases and documents were searched for information to inform and update Chapter 5, including information on production. No information was located to confirm whether or not disulfoton is still used/produced abroad and whether or not that could result in exposure to the U.S. population. It was found that disulfoton is available for purchase for laboratory use from chemical manufacturing companies in the U.S. however it could not be confirmed if this is produced or manufactured abroad.

The following portion was edited in the Profile in section 5.6 (General Population Exposure):

> *"Since disulfoton was canceled by the EPA in 2009 (EPA 2010), the general population in the U.S. is not likely to be exposed to disulfoton, although its use abroad may continue. It is unknown if uses abroad may lead to exposure of the general population as there is insufficient information to confirm its use."*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: It would be useful inserting here more detailed information on the presence of disulfoton in hazardous waste sites, e.g. expanding the comments to Fig. 5.1. It should also be indicated that most if not all the considerations are based on studies which are 20-40-year-old.

RESPONSE: *Information of disulfoton in hazardous waste sites is provided in Figure 5-1 and its preceding paragraph provides general information on the number of hazardous waste sites. Table 5-2 provides more detailed information on levels measured at NPL sites. It is unclear what further information the reviewer is requesting regarding disulfoton in hazardous waste sites.*

In Section 5.5 (Levels in the Environment) additional text was included to indicate when data was collected prior to the cancellation of disulfoton in 2009. The first section in section 5.5.1 (Air) now reads:

> *"No data were located measuring disulfoton in air after its cancellation in 2009 as the concentration in the U.S. is now expected to be lower compared to data from prior to 2009."*

The first two sentences in section 5.5.2 (Water) now reads:

> *"Data measuring disulfoton in water are all from samples taken prior to the 2009 cancellation of disulfoton. Concentrations in water are likely to be much lower than what is reported in the data."*

The first two sentences in section 5.5.3 (Soil and Sediment) now reads:

> *"Only NPL data measured disulfoton in sediment and soils after 2009 and is reported in* ***Error! Reference source not found.****. All other located data are of samples taken prior to the cancellation of disulfoton and levels are expected to now be lower in the U.S."*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: These sections are very comprehensive.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: Table 5.1 on p. 122 reports lowest limit of detection for disulfoton. Most data refer to publications that precede the ban of disulfoton. A few references are dated after 2009. It should be indicated that these values/studies are from foreign countries.

RESPONSE: *Table 5-1 was updated with more recent references as available. Foreign studies were not indicated in the table as it is not necessary for the information being presented, and the format of the table is following the Guidance for the preparation of Toxicological Profiles.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: There is apparently no current exposure to disulfoton given that is it has not been used as an insecticide since 2009, it is not detected in foods as recently as 2017-2019, is relatively rapidly degradable in various media. However, its presence in some hazardous waste sites may imply potential exposure. This may be better discussed.

RESPONSE: *Additional text was added throughout Chapter 5 to further clarify the potential for human exposure from hazardous waste sites, including:*

In the introduction of Chapter 5 the following text was added;

"Due to the potential presence of disulfoton at hazardous waste sites, exposure may be possible for populations who live near these sites."

In section 5.6 (General Population Exposure), the following text was added: "Disulfoton has been detected in soils at multiple hazardous waste sites in the United States, indicating populations living near these hazardous waste sites may be at risk of disulfoton exposure."

Chapter 6. Adequacy of the Database

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

COMMENT: I am not aware of other studies that will add significant different information to the Toxicological Profile for disulfoton.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This section presents a comprehensive summary of data that would be useful. It may be indicated that it is unlikely that such data will ever be available, given the termination of the use of disulfoton.

RESPONSE: *An indication that further data is unlikely to be available was not included in Chapter 6 as these assumptions are beyond the scope of the Profile.*

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

COMMENT: Data seem to be presented in an unbiased fashion.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: I am not aware of any additional regulation of guideline for disulfoton.

RESPONSE: *No revisions were suggested.*

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

COMMENT: No, even the absence of certain regulatory parameters is useful information.

RESPONSE: *No revisions were suggested.*

Minimal Risk Levels (MRLs)

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

COMMENT: The MRL should only be derived when supporting data exist. This has been correctly done in this Toxicological Profile for Disulfoton.

RESPONSE: *No revisions were suggested.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: Appendix A was reviewed to address this issue. It starts with a good definition of MRL, how it is derived, etc. (A1-A2). This is followed by a worksheet for each MRL (acute, intermediate chronic for different routes of exposure). For example, the MRL worksheet for acute oral exposure (pp. A10-A14) starts with the selection of the key adverse effect (correctly, acetylcholinesterase inhibition). Relevant studies are presented in Table A-4. The study by Klaus et al 2005b in rat pups was correctly chosen as it has the lowest value for LOAEL or NOAEL. The study is summarized, and the calculation of the Point of Departure derived from BMD analysis is presented. Uncertainty factors related to animal to human extrapolation ad human heterogeneity were applied (total = 100) to yield the MRL. Similar approaches were used for all other MRLs. The overall approach seems sound and is clearly described.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Additional Comments:

Is disulfoton still produced/used anywhere in the world? If so, could it make its way into the US market as residues in food/feed? Since it would not seem so, would exposure be limited to its presence in some hazardous waste sites?

RESPONSE: *No specific data were located on production of disulfoton abroad. As stated in the Profile, chemical vendors were identified to be selling disulfoton in the U.S. market; however, it cannot be identified where this disulfoton is being produced, and no assumptions can be made on potential for human exposure. Disulfoton is also not permitted for use in the European Union.*

Annotated Comments on the Profile

COMMENT: Section 1.1: The metabolite DEP is diethyl phosphate, not diethyl phthalate.

RESPONSE: *The metabolite DEP was corrected to diethyl phosphate in section 1.1 (Overview and U.S. Exposures). It was confirmed that the correct metabolite was written elsewhere in the profile. Chapter 1 was corrected as follows:*

> *"The urinary metabolites of disulfoton are diethyl phosphate (DEP), diethyl thiophosphate (DETP), diethyl dithiophosphate (DEDPT), and diethyl phosphorothiolate (DEPTh)."*

COMMENT: Section 1.1: DEP and the other listed metabolites can derive by any diethyl organophosphate (as indicated), thus they do not serve as indicators of disulfoton exposure.

RESPONSE: *Section 1.1 (Overview and U.S. Exposures) already indicates these metabolites are not specific to Disulfoton. The profile reads "Although the occurrence of these phosphate esters in human urine may not result specifically from exposure to disulfoton, detection of these metabolites in human urine indicates the possibility of exposure to disulfoton or several other organophosphate insecticides." Therefore, no changes were made as a result of this comment.*

COMMENT: Section 1.1, p. 2 line 273-274: This sentence on lifestyle factors is not clear

RESPONSE: *The comment refers to the following sentence in section 1.2 (Summary of Health Effects): "Additionally, information on lifestyle factors for individuals were not sufficiently assessed; this limitation increases the risk of bias (Futagami et al. 1995; Gomez-Arroyo et al. 2000; Hattori et al. 1982; Wolfe et al. 1978; Yashiki et al. 1990)."*

Lifestyle factors refers to individual habits such as smoking or alcohol consumption, which could either not be controlled or were accounted for in the referenced studies. To clarify, the sentence was edited to the following:

> *"Additionally, information on lifestyle factors, such as smoking, for individuals were not sufficiently assessed; this limitation increases the risk of bias (Futagami et al. 1995; Gomez-Arroyo et al. 2000; Hattori et al. 1982; Wolfe et al. 1978; Yashiki et al. 1990)."*

COMMENT: Section 1.1, p. 3, last sentence: the meaning of "studies on disulfoton on behavior were inconclusive" is not fully clear.

RESPONSE: *The comment refers to the following sentence in section 1.2 (Summary of Health Effects): "Studies are inconclusive on the effect of disulfoton on behavior or functional task performance (Clark and Pearson 1973; Clark et al. 1971; Flucke 1986; Jones et al. 1999; Sheets 1993a)."*

The sentence was edited to better clarify the meaning of the statement. The text now reads the following:

> *"Studies are inconclusive on the whether disulfoton alters behavior or functional task performance in animals (Clark and Pearson 1973; Clark et al. 1971; Flucke 1986; Jones et al. 1999; Sheets 1993a)."*

Appendices

COMMENT:

Appendix A. ATSDR Minimal Risk Levels and Worksheets

Please see comments on p. 2 on MRLs

RESPONSE: *The comment refers to the reviewer's previous comments on the MRLs. No revisions were suggested.*

COMMENT:

Appendix B. Literature Search Framework for Disulfoton

Nothing to add

RESPONSE: *No revisions were suggested.*

COMMENT:

Appendix C. Framework for ATSDR's Systematic Review of Health Effects data for Disulfoton

This is an excellent Appendix that provides a lot of important and useful information, as it is an in-depth analysis of studies on disulfoton. This analysis provides the framework for establishing a level of confidence for each study based on the evaluation of several important and established parameters.

RESPONSE: *No revisions were suggested.*

COMMENT:

Appendix D. User's guide

Theoretically useful but not always very clear. Could benefit of some rewriting.

RESPONSE: *Appendix D is currently in accordance with the Guidance Document. The reviewer did not add specific comments on Appendix D to address.*

COMMENT:

Appendix E. Quick Reference for Health Care Providers

Nothing to add

RESPONSE: *No revisions were suggested.*

COMMENT:

Appendix F. Glossary

Useful list of important terms. I would add the term Insecticide/acaricide, the intended use for disulfoton.

RESPONSE: *The inclusion of additional terms into the Appendix F Glossary will be considered by ATSDR.*

COMMENT:

Appendix G. Acronyms, Abbreviations and Symbols

Useful list of abbreviations and acronyms. Nothing to add.

RESPONSE: *No revisions were suggested.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: Yes. Animal testing is done because animals and humans share many biological targets and processes. Animals have DNA, nervous systems, and many basic receptors and life processes that are analogous to similar potential targets of toxic action in people. In particular both animals and people have nerves that transmit signals to muscles and other nerves by releasing acetylcholine. Once this signaling chemical has been released it must be destroyed in order for additional signals to be accurately transmitted and received across synapses and neuromuscular junctions. Chemicals that interfere with this destruction, such as the subject of the present review, delay the recovery of acetylcholine receptor function.

RESPONSE: *The reviewer's comment generally agrees with the health endpoint conclusions of the Profile. No revisions were suggested.*

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

COMMENT: The experimental systems are adequately described.

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Yes, as far as I can tell without reviewing the cited literature in detail.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going

into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: The chapter is mostly a compilation of observed values from the literature. As far as I can tell, study limitations are adequately addressed

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: The literature on this chemical is extensive, and the mechanism of action very well known, and the acute toxicity observations are ample to support quantitative conclusions.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: The animal species are reasonable to quantify the cholinesterase inhibition toxicity, as the mechanisms involved are shared by a wide variety of animal species.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: I did my own recent literature search and did not find anything notable that should be included in the ATSDR document.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: I did not find any deficiencies in these aspects of the document.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: Seems OK to me.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: Yes. There is only one known significant mechanism of action as far as I known—inhibition of acetylcholinesterase

RESPONSE: *No revisions were suggested.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

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

COMMENT: The ATSDR could have chosen to do its own PBPK modeling, but in context I do not see the purpose that this would serve.

RESPONSE: *The ToxProfile presents any existing PBPK modeling. No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: Yes—I do not know of significant differences that could be elucidated by further analysis.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: No

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: *No reviewer response.*

RESPONSE: *No revisions were suggested.*

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

COMMENT: The key biomarker is acetylcholinesterase inhibition. This is not specific to the subject chemical, as is adequately covered in the document.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Same response as above.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: Interactions with other cholinesterase inhibitors are clear.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: I don't know of any errors in these data as reported in the document.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, as far as I can see.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: As far as I can tell.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: As the chemical is reportedly no longer in use in the U.S., I know of no further relevant information on this topic.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: I know of no further relevant information on this topic.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: I know of no further relevant information on this topic.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: In the light of the fact that the chemical is no longer legal for use in the U.S. I think no further pursuit of this topic is in order.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

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

COMMENT: No.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: No.

RESPONSE: *No revisions were suggested.*

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

COMMENT: No.

RESPONSE: *No revisions were suggested.*

Minimal Risk Levels (MRLs)

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

COMMENT: *No reviewer response.*

RESPONSE: *No revisions were suggested.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

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

COMMENT: The presentation is confusing. An acute MRL is derived, but then there is a discussion under the heading, "**Rationale for Not Deriving an MRL".**

RESPONSE: *The comment refers to Appendix A MRL Worksheet for the acute-duration inhalation MRL. An acute-duration inhalation MRL was not derived using acute-duration inhalation data. Instead, the intermediate-duration inhalation MRL was selected. The rationale for this or "Rationale for Not Deriving an MRL" explains why the acute database was not chosen. This presentation is standardized across all ToxProfiles for instances where a longer term duration MRL is adopted as the shorter term duration MRL.*

Annotated Comments on the Profile

COMMENT: There should be a reference at the end of this sentence so the reader can find the source of these observations. It is possible that the appropriate references are elsewhere in the paragraph, but if so, that is not clear from the current presentation.

RESPONSE: *This comment refers to the following sentence in section 1.2 (Summary of Health Effects): "Clinical findings have measured severely depressed cholinesterase activity and muscarinic effects alongside signs of intoxication including confusion, vomiting and masseteric spasms and other symptoms." This sentence was combined with the preceding sentence for clarity with the appropriate references cited. The following sentence now reads:*

> *"These findings are further corroborated by findings in numerous human case studies where clinic findings have measured severely depressed cholinesterase activity and muscarinic effects alongside signs of intoxication including confusion, vomiting, masseter muscle spasms, and other symptoms (Futagami et al. 1995; Hattori et al. 1982; Savage et al. 1971; Yashiki et al. 1990)."*

COMMENT: It is not clear what it means to "interfere with" neurotransmitter levels in the brain. Increase? Decrease? The document should more clearly convey what the "interference" consists of.

RESPONSE: *This comment refers to the following sentence in section 1.2 (Summary of Health Effects): "In animal studies, disulfoton has been observed to interfere with neurotransmitter levels in the brain, including acetylcholine and catecholamines." The statement should have stated that neurotransmitters accumulated but to further avoid confusion, the sentence was edited to clarify that which specific neurotransmitters are being examined and the observed effect in animal studies. The sentence was edited to the following:*

> *"In animal studies excessive accumulation of acetylcholine and catecholamines has been observed after dosing with disulfoton. ."*

COMMENT: Presumably these are oral doses, but that should be made clear explicitly.

RESPONSE: *This comment refers to the following sentence in section 2.2 (Death): "Deaths occurred on the day of treatment in 4 of 6 female rats at a dose of 2.5 mg/kg and in 1 of 9 female rats at a dose of 1.5 mg/kg, but no deaths occurred in male rats given ≤5.2 mg/kg (Sheets 1993a)." This sentence was edited to clarify that the doses were by oral gavage, and edited to the following:*

"Deaths occurred on the day of treatment in 4 of 6 female rats given a dose of 2.5 mg/kg by oral gavage and in 1 of 9 female rats at a dose of 1.5 mg/kg, but no deaths occurred in male rats given ≤5.2 mg/kg (Sheets 1993a)."

COMMENT: This can be omitted. Effects on human body weight are very rare—I don't recall ever seeing one.

RESPONSE: *The comment refers to the first sentence in section 2.3 (Bodyweight). The statement was kept in the Profile to acknowledge that the literature search included body weight effects but no relevant studies were identified.*

COMMENT: "intussusception" is a very unusual word and will not be understood by many readers. I would suggest omitting it or, at the very least, defining it in a parenthetical expression when it is first used.

RESPONSE: *The comment refers to section 2.6 (Gastrointestinal) under the Dermal subheading. Parentheses were added after the term intussusception to define it for the reader. The sentences was edited to the following:*

"In a 3-week study in which disulfoton was applied to the shorn, unabraded skin of rabbits for 6 hours, 5 days/week, necropsy of the rabbits that died within 2 weeks during treatment (100%) with the high dose of 6.5 mg/kg/day revealed marked intussusception (when one part of the intestine slides inside another part) of the ileum of one female (Flucke 1986)."

COMMENT: This needs to be rewritten. First, what is meant by "the reduction in electron transport elements"? And why is this attributed to "loss of integrity of the membranes and structural alterations in the membrane phospholipids"? And why is this evidence of a "changed conformation of heme protein"? Overall, without further support, this seems to be rather far-out speculation.

RESPONSE: *The comment refers to section 2.9 (Hepatic) under the Oral subheading. The study Fawade and Pawar (1978) was reevaluated as a result of this comment the conclusions of the study were revised, including defining a few terms. Additionally, several of the conclusions are from the study's discussion section and were deleted from the Profile as the assumptions from the authors are not substantially evidenced-based to be included. The statement was edited to the following:*

"Fawade and Pawar (1978) noted disulfoton significantly increased ascorbate-promoted lipid peroxidation and NADPH-driven lipid peroxidation by 13% and 14%, respectively, in mice orally dosed with 0.5, 1, 1.5 or 2 mg/kg/day for 2 days, then all to 1 mg/kg/day for 2 additional days. Study authors suggested that disulfoton or its oxygenated metabolite may have changed the conformation of heme protein thus enhancing lipid peroxidation. Fawade and Pawar (1978) also reported that "hepatic microsomal electron transport elements," defined as cytochrome P-450 and cytochrome b_5, decreased as dose decreased (Fawade and Pawar 1978)."

COMMENT: This makes no sense. What can possibly be meant by "protein degeneration" and "significant circulatory disruptions"? I think these "observations" are best deleted as they are too vague to convey a meaningful type of toxicity. Moreover the absence of an association with a definite dose of the toxicant further renders the observation unhelpful.

RESPONSE: *The comment refers to the beginning of the paragraph under the Dermal subheading in section 2.9 (Hepatic). Following further review of the Kundiev (1967), it was determined that due to poor study quality, there is insufficient information to attribute effects to disulfoton. The study did not specify which effects were caused by which chemical, as several were examined in addition to disulfoton. Overall, the effects are written vaguely and it is difficult to distinguish which effects were seen in rats, cats, or rabbits. The effects reported from the Kundiev (1967) were deleted from the Profile, including sections 2.5, 2.10, 2.11, and 2.15.*

COMMENT: What can possibly be meant by a "lobular pattern in the liver"? This observation is best deleted as not corresponding to any clearly defined manifestation of toxicity.

RESPONSE: *The Flucke (1986) study does not provide further discussion or context to this effect, as it only states that the liver had a "lobular pattern." The study does not specify if this observation is associated with potential health effects. The significance of lobular pattern is inconclusive as some literature suggests it can indicate anemia or lipid accumulation, but generally it appears indicative of a hepatic effect. This effect was still kept in the profile as it can be indicative of an effect, however it was indicated in text and in the LSE table that it was an "enhanced lobular pattern.". Further text was added to clarify the results. The statement was edited to the following:*

> *"Necropsy of these rabbits revealed an enhanced lobular pattern in the liver, however the study authors did not conclude whether this observation is associated with potential health effects (Flucke 1986)."*

COMMENT: It is not at all clear how the authors have concluded that they can dismiss the possibility that the kidney tumors resulted from the disulfoton treatment, or on what basis they are considered "insignificant". Is it meant that there was no statistically significant increase in kidney tumors?

RESPONSE: *The comment refers to the final two sentences under the subheading Oral in section 2.10 (Renal). The study states that the incidence of kidney tumors in the disulfoton treated mice was not statistically significant from controls. The sentence was edited to clarify the details of the study, and now reads :*

> *"The increased kidney weight was thought to be associated with an insignificant increase in the incidence of malignant lymphoma in the kidney. The number of kidneys with malignant lymphoma among exposed mice did not significantly differ from controls, indicating they were not related to disulfoton exposure; the toxicological significance of the increased kidney weight is not clear."*

COMMENT: The meaning of "protein degeneration" and "circulatory disruptions" is completely unclear. Sometimes reports from Eastern Europe cannot be related to toxicological effects that are recognized in the West. That may be the case here. If so, the finding is best ignored.

RESPONSE: *The comment refers to the first two sentences under the subheading Dermal in section 2.10 (Renal) and refers to conclusions from the Kundiev (1967) study. As stated in a previous comment, the conclusions from the Kundiev (1967) study were deleted from the Profile as the study quality was inadequate to be included in the Profile, and results were presented unclearly to definitively attribute reported effects to disulfoton exposure.*

COMMENT: If the doses are unspecified, then I would say that this finding of ill-defined "microscopic changes" is too vague to merit reporting in an ATSDR document.

RESPONSE: *The comment refers to the first three sentences under the subheading Dermal in section 2.11 (Dermal) and refers to conclusions from the Kundiev (1967) study. As stated in the previous comment, the conclusions from the Kundiev (1967) study were deleted from the Profile as the study quality was inadequate to be included in the Profile, and results were presented unclearly to definitively attribute reported effects to disulfoton exposure.*

COMMENT: On what basis? Was absorption measured? If not, I would omit this finding.

RESPONSE: *The comment refers to the first three sentences under the subheading Dermal in section 2.11 (Dermal) and refers to conclusions from the Kundiev (1967) study. As stated in the previous comment, the conclusions from the Kundiev (1967) study were deleted from the Profile as the study quality was inadequate to be included in the Profile, and results were presented unclearly to definitively attribute reported effects to disulfoton exposure.*

COMMENT: The reader needs to be informed what these subtypes are, and what their significance is in terms of overall toxic effects.

RESPONSE: *The comment refers to the following sentence in section 2.15 (Neurological): "The study primarily examined the loss of muscarinic receptors (MR) corresponding to changes in mRNA levels, focusing on m1, m2, and m3 subtypes (Yagle and Costa 1996)." The study was reviewed and a sentence was added immediately after that to specify the roles of the subtypes examined in the Yagle and Costa (1996) study. The following sentence was added in section 2.15 (Neurological):*

> *"The m1 and m3 subtypes activate phosphoinositide hydrolysis, and the m2 subtype is negatively coupled to adenylyl cyclase."*

COMMENT: The reader should be told, at least briefly, what specific measurements lead the author(s) to conclude that there was "an increase in the permeability of spinal cord and brain stem tissues."

RESPONSE: *The comment refers to the following statements in section 2.15 (Neurological): "Dietary exposure of rats and mice to 2.5 mg/kg/day disulfoton for 2 months resulted in an increase in the permeability of spinal cord and brain stem tissues in both species (Clark and Stavinoha 1971). The nature of this change in permeability was not further investigated." The study was reviewed and further detail was added on how the authors evaluated the increased permeability. The following sentence now reads:*

"Dietary exposure of rats and mice to 2.5 mg/kg/day disulfoton for 2 months resulted in an increase in the permeability of spinal cord and brain stem tissues in both species (Clark and Stavinoha 1971). This was evaluated by the permeability of stained brain tissue slices to copper ferricyanide complex. The nature of this change in permeability was not further investigated."

COMMENT: delete "were seen". These words are redundant and confusing in context.

RESPONSE: *The comment refers to the following sentence in section 2.17 (Developmental) that originally read "No treatment-related effects on number of litters, live births, stillborns, and viability were seen after pregnant rats were exposed to 0, 0.038, 0.156, or 0.67 mg/kg/day during gestation days 0-21 (corresponding to doses of 0, 0.102, 0.389, or 1.714 mg/kg/day) during lactation (Sheets 2002)." Agree with the reviewer's suggestion and "were seen" was deleted from the above sentence as follows:*

"There were no treatment-related effects on number of litters, live births, stillborns, or viability in pregnant rats after exposure to 0, 0.038, 0.156, or 0.67 mg/kg/day during gestation days 0-21 (corresponding to doses of 0, 0.102, 0.389, or 1.714 mg/kg/day) during lactation (Sheets 2002)."

COMMENT: This is far too small a group to make any meaningful statement about cancer incidence. I would delete it.

RESPONSE: *The details from the Gómez-Arroyo et al. (2000) were kept in section 2.18 (Cancer). In consideration of the reviewer's comment, the following sentence was added to the section:*

"Given the small sample size, these results cannot be generalized to other populations."

COMMENT: I am not sure this is necessarily correct. The difference between oral and intraperitoneal LD50's could also be at least partly attributable to a delay in gastrointestinal absorption compared to intraperitoneal. This is because delayed absorption could allow some of the administered oral dose to be detoxified before lethal damage was delivered.

RESPONSE: *The comment refers to the following sentence in section 3.1.1 (Absorption): "However, the intraperitoneal LD_{50} values were slightly lower than the oral LD_{50} values, suggesting that gastrointestinal absorption is <100%." ATSDR agrees with the reviewer's comment, and this sentence was deleted as this assumption cannot be definitely stated in the Profile.*

COMMENT: These abbreviations should be defined, possibly in a footnote.

RESPONSE: *The comment refers to the abbreviations "DEP, DETP, and DEDPT" in section 3.1.3 (Metabolism). These abbreviations are defined earlier in the same paragraph for the reader as follows: "diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDPT)".*

COMMENT: What exactly is a "Type 1 spectra"? This must be defined in a parenthetical expression or a footnote, or it will be completely opaque to the reader.

RESPONSE: *The comment refers to the following sentence in section 3.1.3 (Metabolism): "Generally, anticholinesterase insecticides such as disulfoton bind to oxidized cytochrome P-450 to form a disulfoton:cytochrome P-450 complex which usually produces some form of a Type I spectra (Stevens et al. 1973)." "Type I spectra" refers to how the effect was measured. To avoid confusion, this phrasing "which usually produces some form of a Type I spectra" was deleted as it is not necessary to the reporting of effects.*

COMMENT: A reference must be provided for these observations.

RESPONSE: *The comment refers to the following sentence in section 3.1.3 (Metabolism): "These findings underscore the fact that cytochrome P-450 and flavin monooxygenase both have the potential to participate in the oxidation of the disulfoton." It was confirmed that the information was from the Tynes and Hodgson (1985) study, and the reference was added to the end of the sentence.*

COMMENT: It is not at all clear what is meant here by "similar species". One could substitute "mammalian species that have been studied."

RESPONSE: *The comment refers to the first sentence of the final paragraph in section 3.1.3 (Excretion). ATSDR agrees with the reviewer's comment and substituted the phrasing as follows:*

> *"The metabolism of disulfoton appears to be similar among mammalian species studied."*

COMMENT: It is not clear what is meant by "more saturable". Is it that the concentration needed to half-saturate the enzyme is lower (i.e., it has a lower Michaelis constant)? If so this appears to be covered in the next sentence, so there may be an opportunity to consolidate the discussion here.

RESPONSE: *Upon further review of the study, the sentence was deleted as it did not appear to be necessary to the discussion and the meaning of "more saturable" is not further clarified in the study text.*

COMMENT: These abbreviations need to be translated into understandable English for the reader.

RESPONSE: *The comment refers to the abbreviation DEP and DETP in the final paragraph of Section 3.1.4 (Excretion). The abbreviations were defined in the section 3.1.4 as follows:*

> *"White rats given a single dose of radiolabeled disulfoton intraperitoneally eliminated the metabolites phosphoric acid (4.1%), diethyl phosphate (DEP) (61.2%), and diethyl thiophosphate (DETP) (24.8%) in urine as a percentage of excretory hydrolytic metabolites 12 hours after exposure (Bull 1965)."*

COMMENT: I would add "metabolism" so that the line reads "uptake, metabolism, and disposition". Metabolism is often a key aspect of PBPK modeling.

RESPONSE: *ATSDR will consider the reviewer's recommendation in updating this boilerplate text that appears across all Profiles.*

COMMENT: "is" must be changed to "are" here as the subject of the sentence is plural.

RESPONSE: *The comment refers to the final sentence of section 3.1.6 (Animal-to-Human Extrapolations). The reviewer's recommendation was accepted and the sentence was edited as follows:*

> *"It is unknown if these differences between the sexes are seen in humans."*

COMMENT: "effects" must be changed to "affects".

RESPONSE: *The comment refers to the following sentence in section 3.2: "There is insufficient information to determine if effects in children would be similar to effects in adults after acute exposure or long-term low level exposure." The word "effect" was not changed as it is referring to health effects, however the text was revised to the following:*

> *"There is insufficient information to determine if effects in children would be similar to effects seen in adults after acute exposure or long-term low level exposure to disulfoton."*

COMMENT: change "lower levels than adults rats" to "than in adult rats"

RESPONSE: *The comment refers to the following sentence in section 3.2 (Children and Other Populations that are Unusually Susceptible): "Animal studies suggest that younger animals are more susceptible to disulfoton toxicosis than older animals as mortality in weanling rats was seen at lower levels than adults rats (Brodeur and Dubois 1963)." The sentence was edited as follows:*

> *"Animal studies suggest that younger animals are more susceptible to disulfoton toxicosis than older animals as mortality in weanling rats was seen at lower levels when compared to adult rats (Brodeur and Dubois 1963)."*

COMMENT: change "will" to "could". Whether levels are detectable will depend on the dose given, the rate of metabolism and other factors.

RESPONSE: *The comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "Supporting evidence from animal studies indicates that disulfoton exposure will result in detectable levels in the liver (Bull 1965; Puhl and Fredrickson 1975), but monitoring of liver levels in humans would require biopsy." ATSDR agrees with the reviewer's suggestion and "will" was changed to "could" in the sentence.*

COMMENT: The abbreviations DETP and DEDPT need to be defined for the reader in English.

RESPONSE: *The comment refers to the abbreviation DETP and DEDPT in section 3.3.1 (Biomarkers of Exposure). The abbreviations were defined in the sentence as follows:*

> *"Specimens of urine collected from 31 locations across the United States, comprising the sample areas of the National Health and Nutrition Examination Survey (NHANES) from 2011-2012,*

reported detection (detection limit 0.07 ng/mL) of diethyl thiophosphate (DETP) at a frequency of 71% and diethyl dithiophosphate (DEDPT) at a frequency of 4% of those tested (CDC 2012)."

COMMENT: HMMA must be defined for the reader, possibly in a parenthetical expression.

RESPONSE: *The comment refers to the abbreviation HMMA in section 3.3.1 (Biomarkers of Exposure). The abbreviation was defined for the reader in the sentence as follows:*

"The major metabolite of catecholamine metabolism, 4-hydroxy-3-methoxymandelic acid (HMMA), was also detected in the urine from rats given acute doses of disulfoton (Wysocka-Paruszewska 1971)."

COMMENT: This statement does not appear to be supported by material elsewhere in the paragraph. I suggest it be deleted. Also, "Furthermore" at the beginning of the next sentence can be deleted.

RESPONSE: *The comment refers to the last paragraph of section 3.3.1 (Biomarkers of Exposure). ATSDR agrees with the reviewer's comment and incorporated the suggested edit as follows:*

"These changes are not specific for disulfoton exposure, and these subtle liver effects require invasive techniques in humans to obtain liver tissue for performance of these enzyme assays."

COMMENT: I don't think this speculation is needed. I would delete it.

RESPONSE: *The comment refers to the following sentence in section 3.3.2 (Biomarkers of Effect): "This enzyme appears to be a useful biomarker of hepatic function in rats exposed to disulfoton, but may not be a useful biomarker in humans." ATSDR agrees with the reviewer's comment and deleted this sentence.*

COMMENT: "HMMA"—spell out what this abbreviation stands for the first time it is used.

RESPONSE: *The comment refers to the abbreviation HMMA in section 3.3.2 (Biomarkers of Effect). The abbreviation HMMA was defined as follows:*

"The major metabolite of catecholamine metabolism, 4-hydroxy-3-methoxymandelic acid (HMMA), was also detected in the urine from rats given acute doses of disulfoton (Wysocka-Paruszewska 1971)."

COMMENT: Change to "However how an increase in gamma-enolase mRNA indicates toxicity is unclear (Matsuda et al. 2000).

RESPONSE: *The comment refers to the final sentences in section 3.3.2 (Biomarkers of Effect). ATSDR agrees with the reviewer's comment and the sentence was edited to the following:*

"However, how an increase in gamma-enolase mRNA indicates toxicity is unclear (Matsuda et al. 2000)."

COMMENT: I think it is highly doubtful that different forms of cytochrome P450 are interconvertible. Even though some researcher has evidently said it, I can't see how it is possible. Different P450s are different protein molecules, each with its own sequence of amino acids. I would suggest deleting the dubious implication that they are somehow interconvertible.

RESPONSE: *The comment refers to the discussion of effects reported in the Pawar and Fawade (1978) study in section 3.4 (Interactions with Other Chemicals). The Pawar and Fawade (1978) was reviewed and it was confirmed that the study states "incontrovertible forms" of cytochrome P450. Per the reviewer's suggestion, the referenced statement was deleted as it appears likely the statement from the study is outdated.*

COMMENT: Just what is a "type 1 substrate"? This unfamiliar term must be defined for the reader.

RESPONSE: *The comment is reference to the phrase "type 1 substrate" in section 3.4 (Interactions with Other Chemicals) in reference to ethylmorphine. This statement was deleted. Type 1 substrate was wording used by the study authors to describe the substrate used in the experimental procedures, however the study does not define what this means.*

COMMENT: The sentence starts with "These electron transport components…" However the subject of the previous sentence is "Nickel chloride, cobalt chloride, or cycloheximide…"

Doubtless the reference is actually to the cytochromes and possibly heme, but this must be clarified in order to avoid confusion.

RESPONSE: *The comment refers to the fifth paragraph in section 3.4 (Interaction with Other Chemicals). This paragraph was revised for clarity as follows:*

"The levels of electron transport chain components (cytochrome b, cytochrome c reductase, and total heme) in rats were lowered by administration of nickel chloride, cobalt chloride, or cycloheximide (Fawade and Pawar 1983). When given a single dose of disulfoton, the electron transport components were further decreased in rats pretreated with nickel chloride or cobalt chloride."

COMMENT: It is not at all clear what is being referred to as "these inhibitors"

RESPONSE: *The comment refers to the same paragraph as the previous comment and the edits provided above apply to this comment as well. To clarify based on this comment the statement in the profile now reads:*

> *"The levels of electron transport chain components (cytochrome b, cytochrome c reductase, and total heme) in rats were lowered by administration of metabolic inhibitors, nickel chloride, cobalt chloride, or cycloheximide (Fawade and Pawar 1983). When given a single dose of disulfoton, the electron transport components were further decreased in rats pretreated with nickel chloride or cobalt chloride. Data from this study suggests an additive effect by disulfoton (Fawade and Pawar 1983). In a separate experiment, an additive effect between disulfoton and the tested metabolic inhibitors was suggested by the decrease in ethylmorphine N-demethylase and acetanilide hydroxylase activities when rats were given an inhibitor followed by disulfoton. In*

another experiment, the inhibitors decreased the activity of delta-aminolevulinic acid synthetase, but this decrease was reversed when disulfoton was administered (Fawade and Pawar 1983)."

COMMENT: The fact that a small decrease was produced does not necessarily imply that the effect is additive. This needs to be clarified.

RESPONSE: *The comment refers to the following sentence in section 3.4 (Interaction with Other Chemicals): "Ethylestrenol alone caused a small decrease in cholinesterase activity, and therefore resulted in an additive effect." The final statement in the sentences referenced was deleted as ATSDR agrees with the reviewer that an additive effect is not implied from the observed effect. The sentence was edited to the following:*

"Ethylestrenol alone caused a small decrease in cholinesterase activity."

COMMENT: These statements must be supported by one or more references—probably using some of the references given in Table 4.2.

RESPONSE: *The comment refers to the first sentence of section 4.2 (Physical Chemical Properties). References were added to the support the statement as follows:*

"Pure disulfoton is a colorless oil with low volatility and low water solubility, but is readily soluble in most organic solvents and fatty oils (Bowman and Sans 1983; Spencer 1982). "

COMMENT: Disulfoton production in the U.S. is expected to have ceased since, however disulfoton appears available for purchase through various chemical vendors, but no information on where it is produced is available (PubChem 2020). Break this into at least two separate sentences.

RESPONSE: *This comment refers to section 5.2.1 (Production). The paragraph was revised for clarity to the following:*

"Disulfoton production in the United States is expected to have ceased due to its cancellation for use as a pesticide by EPA in 2009. Disulfoton can be produced commercially by a reaction of the sodium salt of O,O'-diethylhydrogen phoshorodithioate with 2-chloroethylthioethyl ether (VonRumker et al. 1974). ***No information is available in the Toxics Release Inventory (TRI) database on facilities that manufacture or process disulfoton because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (****EPA 2005****).*** *Information on current production volume is also not available in EPA's Chemical Data Reporting database (CDR 2016). Following its cancellation by the EPA in 2009, remaining stock was allowed to be sold and distributed by registrants until 2011 (EPA 2010). Disulfoton is listed as available for purchase through various chemical vendors however there is insufficient information to determine where it is being produced (PubChem 2020). According to the National Pesticide Information Retrieval System, there are currently no federally active products that contain disulfoton (NPIRS 2020)."*

COMMENT: It is unusual to quote a range starting from the higher end to the lower end. I suggest that the ends of the range be double-checked, and then put into the usual order of lower end first followed by the higher end.

RESPONSE: *This comment refers to the final sentence in section 5.2.2 (Import/Export). ATSDR agrees with the reviewer and numbers were rewritten as follows:*

> *"An analysis of shipping records from 2001 to 2003 indicated that U.S. exports of disulfoton in that time period ranged from 118,573 to 288,054 lbs. (Smith et al. 2008)."*

COMMENT: remove initial capital "C" in "Cole"

RESPONSE: *The comment refers to the word "cole" in section 5.2.3 (Use). The reviewer's recommended edit was incorporated (changed to a lowercase "c").*

COMMENT: Since disulfoton no longer has permitted uses, its "efficacy" in those uses seems irrelevant.

RESPONSE: *The comment refers to the following sentences in section 5.4.1 (Transport and Partitioning): "As the sulfoxide and the sulfone metabolites are the major toxicants in the foliage of potato plants grown in disulfoton-treated soil, this reduction in toxicant residues over time can be expected to reduce insecticide efficacy." The statement was kept as discussing the efficacy is still relevant as it is still technically possible that the public may still use a product that was sold prior to its cancellation. Disulfoton pesticide stocks were permitted to be sold up until 2011.*

COMMENT: Based on the diversity of organisms (with diverse fat content, among other variables) this 100-110 seems a misleadingly narrow range for a bioconcentration factor. Probably it should be represented as a central estimate for a factor that undoubtedly spans at least many fold if not an order of magnitude.

RESPONSE: *The comment refers to the second sentence under the Other Media subheading in section 5.4.1 (Transport and Partitioning). The Kenaga (1980) study was reviewed and the sentence was revised in order to give better context on the BCF range provided by the study, as follows:*

> *"Based on these regression equations, the central estimated BCF for disulfoton in aquatic organisms is 100-110; BCF can vary up to two orders of magnitude between species and life stages (Kenaga 1980). "*

COMMENT: This concluding sentence of the paragraph seems contradicted by the previous sentences, which quote just such reaction rates. Some rewrite is needed to remove the apparent contradiction.

RESPONSE: *The refers to the final sentence under the Air subheading in section 5.4.2 (Transformation and Degradation). This sentence was deleted as it appears outdated considering the new data added to the paragraph.*

COMMENT: The special conditions of this experiment (e.g. 100 µm hydrogen peroxide) render the results of dubious relevance

RESPONSE: *The comment refers to the following sentence in section 5.4.2 (Transformation and Degradation): "When a 13 µm solution of disulfoton was exposed to October sunlight (Davis, California) in the presence of 100 µm hydrogen peroxide, 49% of the insecticide disappeared in 10.2 days due to reaction with hydroxyl radicals (Draper and Crosby 1984)." The statement was kept in as the special condition of the study are adequately considered in the proceeding sentence as follows: "Since eutrophic water samples of the same type studied generate hydrogen peroxide levels 30 µM or lower, the rate of this reaction will be slower in natural surface water (Draper and Crosby 1984)."*

COMMENT: Earlier in the paragraph, loss figures were given in terms of half lives. For this sentence, however, a shift is made to "persistence", which is not defined mathematically. Either the "persistence" numbers should be eliminated or converted to terms that are comparable to the half life estimates given earlier in the paragraph.

RESPONSE: *The comment refers to the following sentence in section 5.4.2 (Transformation and Degradation): "The estimated persistence of disulfoton in soil varied between 28 and >64 days (Belanger and Hamilton 1979; Clapp et al. 1976; Jury et al. 1987b; Kearney et al. 1969)." The cited studies were reviewed and confirmed to specifically report on "persistence,." To respond to this comment a definition of persistence in the context of the study was defined as follows:*

> *"The estimated persistence of disulfoton, defined as the concentration of disulfoton remaining elevated or constant in soil, varied between 28 and >64 days (Belanger and Hamilton 1979; Clapp et al. 1976; Jury et al. 1987b; Kearney et al. 1969). "*

COMMENT: Perhaps in a footnote, the text should let the reader know what the numbers to the right of the "±" signs are—standard deviations? --standard errors?

RESPONSE: *The comment refers to the use of the symbol "±" in section 5.5.4 (Other Media). In the text, further context was added to assist the reader in understanding what the symbol refers to as follows:*

> *"Mean disulfoton concentrations and standard deviations were 20±17 ng/g in liver, 17±16 ng/g in gonads, 7±8 ng/g in muscle, and 7±4 ng/g in gills."*

COMMENT: Not detected in how many samples? It would be helpful for the reader to have this information on the number of samples taken to judge the significance of the non-detection.

RESPONSE: *The comment refers to the discussion of testing results from the Gelardi and Mountford (1993) study in section 5.5.4 (Other Media). The Gelardi and Mountford (1993) study was reviewed to add in details on how many samples were tested. The sentence was edited as follows:*

> *"In a 1993 study of pesticide residue contamination of processed infant formula, disulfoton was not detected (detection limit <0.02 µg/g [ppm]) in 32 milk-based and 25 soy-based infant formulas (Gelardi and Mountford 1993)."*

COMMENT: The document should let the reader know what each of these chemicals are, at least one first use of each abbreviation.

RESPONSE: *The comment refers to the abbreviations DEP, DETP, DEDPT, DEPTh in section 5.6 (General Population Exposure). These abbreviations are previously defined earlier in the Profile, but full definitions were added as follows:*

> *"The urinary metabolites of disulfoton are diethyl phosphate (DEP), diethyl thiophosphate (DETP), diethyl dithiophosphate (DEDPT), and diethyl phosphorothiolate (DEPTh)."*

COMMENT: This is not a complete sentence. I suggest it be changed to "However, it is possible that remnants of previously allowed pesticide products may still be available for use."

RESPONSE: *The comment refers to the final paraph in section 5.7 (Populations with Potentially High Exposures). The entire paragraph was restructured per this reviewer's comment and reviewer 3's comments as follows:*

> *"Children may receive higher disulfoton doses from ingestion or dermal exposures if they play in contaminated soils near hazardous waste sites or in soils where a disulfoton pesticides was applied, however this is less likely as disulfoton pesticides were canceled in the U.S. in 2009. Previously allowed pesticides containing disulfoton may still be in circulation."*

Comments provided by Peer Reviewer #3

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: I agreed that only those effects known to occur in humans, as reported.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: The effects only observed in animals not likely to be of concern to humans since Disulfoton is a manmade chemical, and it was canceled by the Environmental Protection Agency (EPA) in 2009 for use as a pesticide. Therefore, the potential for human exposure is low, and these effects observed in animal experiments were at relatively high doses. Human exposure to such high exposure is not likely to happen.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer believes that exposure conditions been adequately described.

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: This reviewer believes that health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Human studies identified in the text are limited duet to the lack of exposure data, or a short period of exposure, and these confounding factors have not able to be controlled. These major study limitations were sufficiently described in the text without going into lengthy discussions.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: This reviewer believes that adequately designed animal studies identified in the text.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: This reviewer believes that the animal species were appropriate for the most significant toxicological endpoint of the study.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Adequate attention has been paid to extrapolate dose-response relationships from animals to humans.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: This reviewer is not aware of any studies that are not included in the profile.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: This reviewer is not aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: NOAELs and/or LOAELs were identified for most of the studies if the data allow.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: This reviewer agrees with the categorization of "less serious" or "serious" for the effects cited in the LSE tables.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: This reviewer believes that all possible mechanisms of action have been discussed within their relevant health effect section.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: This reviewer believes the conclusions are appropriate, given the overall database.

RESPONSE: *No revisions were suggested.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: This reviewer believes there is an adequate discussion of absorption, distribution, metabolism, and excretion of the substance.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer believes all available pharmacokinetic/pharmacodynamic models and supporting data have been presented.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: This reviewer believes there is an adequate discussion of the differences in toxicokinetics between humans and animals since there are not enough human data for the toxicokinetic analysis. There is an adequate discussion of the relevance of animal toxicokinetic information for humans.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: This reviewer believes there is no additional data relevant to child health and developmental effects that have not been discussed in the profile.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: No data were located that identify subpopulations of humans more susceptible to the toxic effects of disulfoton. Therefore, it is impossible for this profile further to discuss the populations at higher risk of susceptibility.

RESPONSE: *No revisions were suggested.*

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

COMMENT: The presence of disulfoton and/or its metabolites in urine is considered a reliable biomarker of disulfoton exposure. Inhibition of red blood cell acetylcholinesterase activity or serum cholinesterase activity with or without concomitant neurological signs is a common indicator of organophosphate exposure, but it is not a specific biomarker for disulfoton.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Depression of cholinesterase activity can be a biomarker for disulfoton, reflecting the degree of synaptic cholinesterase inhibition in nervous tissue. However, this biomarker is not specific for disulfoton.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: This reviewer believes there is an adequate discussion of the interactive effects with other substances, but not aware that if the discussion concentrate on those effects that might occur at hazardous waste sites since the level of disulfoton is low at hazard waste sites.

RESPONSE: *The comment refers to Section 3.4 Interactions with Other Chemicals. There isn't information to warrant a discussion of effects of disulfoton interacting with other chemicals at hazardous waste sites. No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: No comment from the reviewer.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: This reviewer believes the information provided in the chemical and physical properties tables is right.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer is not aware there is information on the various forms of the substance.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: Yes, there is information on production, import/export, use, and disposal of the substance.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: The text appropriately traced the substance from its point of release to the environment until it reaches the receptor population. The text provides sufficient and technically sound information regarding the extent of occurrence at NPL sites. This reviewer has no other relevant information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: This reviewer believes that the text covers pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media. This reviewer has no other relevant information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: This reviewer believes the text provides information on levels monitored or estimated in the environment, including background levels.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: This reviewer believes the text describes sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures. This reviewer agrees with the selection of these populations.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

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

COMMENT: This reviewer does not know of other studies that may fill a data gap.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer agrees with the identified data needs.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer believes the current description presented in a neutral, non-judgmental fashion.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: This reviewer is not aware of any additional regulations or guidelines that should be included.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer believes this a well-summarized Profile, and there is no need to remove anything from the current text.

RESPONSE: *No revisions were suggested.*

Minimal Risk Levels (MRLs)

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

COMMENT: This reviewer agrees that insufficient data for the derivation of an MRL for chronic inhalation exposure. All other MRLs were reasonably calculated based on the values of NOAEL.

RESPONSE: *No revisions were suggested.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: This reviewer agrees with the proposed MRL values. Each component of the total uncertainty factor was appropriately derived based on reported studies.

RESPONSE: *No revisions were suggested.*

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

COMMENT: This reviewer does not have any specific comments on additional aspects which are needed to be addressed.

RESPONSE: *No revisions were suggested.*

Appendices

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

COMMENT: No comment from the reviewer.

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

COMMENT: Need to clarify if the developmental effects on offspring are the direct effect of inhibition of cholinesterase or indirect impact on the developmental process.

RESPONSE: *The comment refers to the Taylor (1965a) summary of developmental effects in Section 1.2. The study was reviewed and there was no indication that developmental effects resulted from depressed acetylcholinesterase activity but rather from exposure to disulfoton and its effect on the developmental process. The sentences were edited as follows:*

"Additionally, in a multi-generational exposure study, third-generation offspring had significantly depressed red blood cell AChE activities (Taylor 1965a). Swelling of the liver, mild nephropathy, and juvenile hypoplasia of the testes were also observed, likely resulting from exposure during gestation (Taylor 1965a)."

COMMENT: It is confusing that decreased sperm was observed in F1 females.

RESPONSE: *The comment refers to the reproductive effect described in entry 42 of Table 2-2 Levels of Significant Exposure to Disulfoton – Oral. The effect states decreased "sperm-positive females" which indicates females who are presumed pregnant after mating based on the presence of sperm in the vagina. The entry was modified with the added text underlined*

"decreased sperm-positive (less mating) F0 (21-33%) & F1 females (22-33%); decreased maternal F1 weight during gestation (8-12%) and lactation (10-12%), and maternal F0 weight during lactation (4-8%) compared to control; decreased litter counts, viability, lactation indices, and increased stillbirths among all litters at 0.09 mg/kg/day."

COMMENT: Sentence is not clear, and needs to be revised.

RESPONSE: *The comment refers to the Chapter 2 LSE table footnote describing acetylcholinesterase activity. ATSDR agrees with the reviewer's comment and the footnote was revised in all tables to the following:*

"Red blood cell and brain acetylcholinesterase activity are assessed by comparing the activity of exposed groups to study controls and assessing whether acetylcholinesterase was inhibited by the chemical of interest. ATSDR classifies a NOAEL as <20% inhibition; a LOAEL is classified as 20-59% inhibition; and a serious LOAEL (SLOAEL) is classified as >59% inhibition. If acetylcholinesterase activity is inhibited by 20-59% but is accompanied with clinical signs of cholinergic toxicity, it may be classified as a SLOAEL. "

COMMENT: Sentence needs to be revised.

RESPONSE: *Similar to the previous comment, this comment refers to the Chapter 2 LSE table footnote describing acetylcholinesterase activity. See previous comment for edits made to the footnote for all LSE tables.*

COMMENT: Grammarly issue.

RESPONSE: *The comment refers to the final paragraph in section 5.7 (Populations with Potentially High Exposures). Reviewer 2 comments were provided for the same paragraph, which was edited as follows:*

"Children may receive higher disulfoton doses from ingestion or dermal exposures if they play in contaminated soils near hazardous waste sites or in soils where a disulfoton pesticide was

applied, however this is less likely as disulfoton pesticides were canceled in the U.S. in 2009. Previously allowed pesticides containing disulfoton may still be in circulation."