DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE (TRIBUFOS)

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

August 2017

Peer reviewers for the third pre-public comment draft of the Toxicological Profile for S,S,S-Tributyl Phosphorotrithioate (Tribufos) were:

Edna F. Pereira, Ph.D.
Associate Professor
Primary Appointment: Epidemiology & Public Health
Secondary Appointments: Pharmacology
University of Maryland School of Medicine
655 West Baltimore Street
Baltimore, MD 21201

Michael Eddleston, M.D., Ph.D.
Department of Pharmacology, Toxicology, and Therapeutics
University/BHF Centre for Cardiovascular Science
University of Edinburgh
Edinburgh, UK

Richard A Fenske, Ph.D., MPH
Associate Chair, Environmental and Occupational Health Sciences
Professor, Environmental and Occupational Health Sciences
University of Washington
Box: 357234
1959 NE Pacific Street
Seattle, WA 98195

Reviewers submitted files of the draft Toxicological Profile for Tribufos with embedded comments and/or suggested text revisions. Suggestions which were editorial and/or stylistic nature were addressed at the discretion of ATSDR. Other suggestions that required a formal response are identified by Chapter, Section, page, and/or line number associated with the file of annotated comments provided by a particular Reviewer. For example, Reviewer #1 submitted a comment on P2, L25-30 which refers to page 2 lines 25-30 of the file submitted by Reviewer #1.

Comments provided by Reviewer #1:

General Comment

The Reviewer stated "This is a well written, concise summary of the available toxicological information for tribufos. I have noted within the document the occasional typo or undefined abbreviation. My review, based on my clinical and preclinical experience of OP poisoning, has focused on sections 1 to 3 but covered all sections. I note its similarity with Toxicological Profile # 86 for diazinon."

RESPONSE: *A search was performed to identify and rectify typos and undefined abbreviations.*

Comments on key issues identified by Reviewer #1

Issue #1: "Information on organophosphate induced delayed polyneuropathy (OPIDN), the role of OP induced inhibition of neurotoxic esterase (NTE) activity in its development, and the potential role of tribufos as an NTE inhibitor in causing OPIDN."

COMMENT: "No mention is made of OPIDN in section 2.2, Summary of health effect, or in section 3.2, Discussion of health effects, yet this may be a key effect of tribufos exposure in humans. Instead only the acute cholinergic syndrome is discussed. This absence of OPIDN in the review contrasts with all textbook chapters on OP toxicity."

RESPONSE: *Brief discussions of the results from studies of hens administered tribufos were added to Sections 2.2 and 3.2 of the profile, along with the following statement: "Although studies of hens are useful for hazard identification, applicability of the dose-response in hens to humans is uncertain."*

COMMENT: "Tribufos induced neuropathy is alluded to in section 3.2.1.4, neurological effects, yet there has been no mention of such neuropathy in the previous sections on health effects. Clearly information on OPIDN needs to be added to sections 2.2 and 3.2."

RESPONSE: *See previous response.*

COMMENT: "Section 3.2.1.4 states that since studies of hens are not useful for risk assessment, they will not be discussed. While I accept that the dose response in humans may well differ from that in hens [1], I do not believe that this subject should be completely ignored. Indeed, it is the sole reason that tribufos has had its registration withdrawn in Australia (see the Australian NRA report at (http://apvma.gov.au/sites/default/files/publication/14831-tribufos-special-review-report.pdf)."

RESPONSE: *The statement in question was deleted. Brief discussions of the results from studies of hens administered tribufos were added to Sections 2.2, 3.2.1.4, 3.2.2.4, and 3.2.3.4 of the profile, along with the following statement: "Although studies of hens are useful for hazard identification, applicability of the dose-response in hens to humans is uncertain." Sources cited for the hen studies were Abou-Donia et al. (1979) and Francis et al. (1985).*

COMMENT: "Lotti and Johnson have argued that studies in hens are relevant for understanding whether particular OP compounds will cause OPIDN in humans [2]. Although this original paper did not assess tribufos, the authors have subsequently assessed this OP compound and consider it likely to inhibit

NTE in humans (see the Australian NRA report). The human experience of poisoning with tribufos is very limited, as illustrated by this review. Therefore it is not surprising that neuropathy has not been reported. However, the hen studies clearly indicate that human exposure may cause OPIDN."

RESPONSE: *The potential for tribufos-induced OPIDN in humans is not in question. The major issue with the results from hen studies is the uncertainty in applicability of the dose-response characteristic in hens to the human experience.*

COMMENT: "NTE inhibition is not ignored completely. It is discussed later on in the report - for example, in section 3.2.3.4, Neurological effects, and in Section 2.3, reporting that 50% NTE inhibition has been described by Lotti in workers exposed to tribufos. It is also discussed in section 7.3.1, Identification of data needs, but without context. OPIDN and NTE inhibition should be included within this review at every point where pathogenesis and clinical features are discussed. The later sections on biomarkers etc. can then build on this information."

RESPONSE: Information regarding tribufos-induced OPIDN in hens is included in Sections 2.2 and 3.2. A discussion of mechanisms of OPIDN was added to Section 3.5.2, along with a statement that tribufos-induced OPIDN has not been observed in exposed humans.

Issue #2: "The description of the cholinergic symptoms of OP poisoning in section 2.2 (repeated in section 1, public health summary, and section 3) is rather long, excessive and not particularly helpful. For the public readership of section 1, in particular, it should be simplified."

COMMENT: "In section 1, these clinical features are described as symptoms, yet many are not symptoms but signs. Patients do not complain of constricted airways or low blood pressure, for example. The public may also not understand words such as urinary incontinence, twitching, insomnia, et cetera. Many of my patients would not understand these words. The text would benefit from simplification."

RESPONSE: *The text was shortened somewhat and the wording of some effects was simplified.*

COMMENT: The Reviewer noted that the toxicological profile states "Severe AChE inhibition leads to muscarinic cholinergic features such as excessive glandular secretions (salivation, lacrimation, rhinitis), miosis, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia." The Reviewer noted that these effects "also occur after much milder poisoning. Patients with mild poisoning in my clinical experience commonly present with constricted pupils and excess secretions, without the other listed features." The Reviewer suggested that "it might be more accurate and probably more useful to state in all the relevant sections that very high levels of tribufos (or severe AChE inhibition) result in excess sweating, very small pupils, unconsciousness, and difficulty with breathing that requires medical assistance, rather than a long list of muscarinic, nicotinic, and central features. These are the key features of OP compound toxicity that will be seen in all patients with severe poisoning. Many of the other features do not occur in all patients and may occur after exposure to other poisons. Convulsions are not common features of severe OP pesticide poisoning."

RESPONSE: *See previous response.*

Issue #3: "The exposure biomarker discussion in section 1 focuses on urine testing for the OP compound. Later in the review (e.g., sections 3.8 and 3.12.2, Biomarkers of exposure and effect),

the value of measuring acetylcholinesterase and/or butyrylcholinesterase activity is downplayed or dismissed. While reduced activity can result from recent exposure to any compound inhibiting these cholinesterases, normal activity can exclude even mild recent exposure to tribufos."

COMMENT: "Identifying whether a single butyrylcholinesterase measurement is reduced or normal can be difficult because of wide inter -individual variability. However, repeat measurements after removal from potential exposure can inform whether the first value was reduced or not. Variation in acetylcholinesterase activity is less important because it should be standardised against blood haemoglobin concentrations. I think the report would benefit from a fuller discussion of the use of butyrylcholinesterase and acetylcholinesterase activity measurements in assessing recent exposure. Once the OPIDN and NTE issue is more fully discussed in the review (see point 1 above), the role of lymphocyte NTE assays for detecting exposure should also be similarly discussed. Clinicians seeing a patient at risk of exposure to tribufos should immediately measure at least butyrylcholinesterase and probably acetylcholinesterase. This should be reflected in the review."

RESPONSE: *Information regarding the use of butyrylcholinesterase (BuChE), acetylcholinesterase (AChE), and neurotoxic esterase (NTE) as biomarkers of effect to substances such as tribufos was added to Section 3.8.2.*

Issue #4: "The literature cited in section 3.11, Methods for reducing toxic effects, is old and out of date."

COMMENT: "I have suggested keeping one book chapter and added two more recent reviews based on extensive clinical experience and research on organophosphorus insecticide poisoning. They should be more relevant to this review. I have further revised the order and edited the text of the subsequent treatment section to reflect more current views on management of OP poisoning."

RESPONSE: *More recent documents regarding reducing toxic effects of organophosphorus compounds such as tribufos were cited (Eddleston 2015; EPA 2013b). Suggested revisions and additions to the text in this section of the toxicological profile were incorporated for the most part.*

Issue #5: "The defoliant merphos (S,S,S-tributyl trithiophosphite) is metabolised to S,S,S-tributyl phosphorotrithioate (DEF), at least in the environment.

COMMENT: "Since there is a small amount of data on human exposure to merphos, I wonder whether it might be worth discussing it within this report? A similar situation occurs with carbosulfan and carbofuran, the latter being the metabolite of the former. Data from carbosulfan poisoning is helpful for understanding carbofuran poisoning."

RESPONSE: *The available human data regarding merphos are included in Chapter 2, Relevance to Public Health, (inhalation MRL), Chapter 4, Physical and Chemical properties, Chapter 6, Potential for Human Exposure-Overview, -Air Levels, and Chapter 7, Analytical Methods- Environmental Samples,- Methods of the toxicological profile.*

Comments on Charge Questions and Statements from the Guidelines for Peer Review of ATSDR's Toxicological Profiles

Charge question and statement: "Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?"

COMMENT: "I have not been able to find any relevant data that have been missed."

RESPONSE: *No response is necessary.*

Charge question and statement: "Are there any general issues relevant to child health that have not been discussed in the profile and should be."

COMMENT: "No."

RESPONSE: *No response is necessary.*

CHAPTER 1. PUBLIC HEALTH STATEMENT

COMMENT: "With the caveat raised above concerning the likely clinical presentation of tribufos poisoning, I found the section to be well written and likely to be at the level understandable by an average citizen."

RESPONSE: *As stated previously, the text regarding clinical presentation of tribufos poisoning was shortened somewhat and the wording of some effects was simplified.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

COMMENT: "Again with the caveat concerning OPIDN raised above, the reported effects are likely to be seen in affected humans. However, there is a gross lack of data from humans, likely due to infrequent exposure. The exposure conditions have been adequately described."

RESPONSE: *Information regarding OPIDN was added to Section 2.2.*

CHAPTER 3. HEALTH EFFECTS

COMMENT: "In the absence of the primary reports themselves, I found this to be useful summary of the data from animal research. I found the conclusions drawn by the authors to be appropriate and the data to be accurately reflected in text, tables, and figures. The calculation of NOAELs and LOAELs appeared appropriate from the data presented. The data on tribufos toxicity, even in animals, appears limited but I am not aware of any additional studies. I was happy with the categorisation of less serious versus serious."

RESPONSE: *No response is necessary.*

COMMENT: "The toxicokinetics section appeared adequate in light of the complete lack of data from humans, which hinders any discussion of the relevance of the animal data to humans."

RESPONSE: *No response is necessary.*

COMMENT: "The biomarker sections could be improved for clinician users of this review. Although the various esterase activities are not specific to tribufos, normal activity indicates a lack of recent exposure to this compound. Therefore they have clinical utility."

RESPONSE: *The section was revised to note that a biomarker of exposure would be the presence of tribufos in the blood or urine. The following statement was also added: "Decreased activities of the enzymes BuChE, AChE, and/or NTE in blood serve as biomarkers of effect from exposure to substances (including tribufos) that inhibit these enzymes. However, decreased activity of these enzymes is not a biomarker specific to tribufos."*

COMMENT: "The management/reducing toxic effects section has been rewritten."

RESPONSE: *See responses to specific comments on annotated pages (identified as Section 3.11 Methods for Reducing Toxic Effects).*

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

COMMENT: "No comments."

RESPONSE: *No response is necessary.*

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

COMMENT: "No comments."

RESPONSE: *No response is necessary.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

COMMENT: "This section is outside my experience but reads well."

RESPONSE: *No response is necessary.*

CHAPTER 7. ANALYTICAL METHODS

COMMENT: "Issues raised in this chapter are similar to those raised above concerning biomarker analysis."

RESPONSE: *See responses to specific comments on annotated pages (identified as Section 7.3.1 Identification of Data Needs).*

CHAPTER 8. REGULATIONS, ADVISORIES, AND GUIDELINES

COMMENT: "No comments."

RESPONSE: *No response is necessary.*

CHAPTER 9. REFERENCES

COMMENT: "Additional references are suggested both in the text of this response and also within the annotated document."
"Abou-Donia MB. Toxicokinetics and metabolism of delayed neurotoxic organophosphorus esters. Neurotoxicology 1983; 4: 113-29."
"Lotti M, Johnson MK. Neurotoxicity of organophosphorus pesticides: predictions can be based on in vitro studies with hen and human enzymes. Archives of toxicology 1978; 41: 215-21."

RESPONSE: *Information regarding OPIDN in hens was added to the toxicological profile. Sources cited were Abou-Donia et al. (1979) and Francis et al. (1985). Husain (2014) was cited as a source of information regarding possible mechanisms of OPIDN. These sources were considered more appropriate than the suggested sources (Abou-Donia et al. 1983; Lotti and Johnson 1978).*

Unpublished studies

COMMENT: "I am unaware of any unpublished studies."

RESPONSE: *No response is necessary.*

Specific Comments on Annotated Pages of the Toxicological Profile for Tribufos

CHAPTER 1. PUBLIC HEALTH STATEMENT

COMMENT: P2, L25-30: Regarding the list of symptoms caused by exposure to very high levels of tribufos, the Reviewer stated: "This is a long list – do people know what constriction of the airways or urinary incontinence are? Many of my patients would not know. Might it not be easier to say that very high levels of tribufos would result in excess sweating, very small pupils, sometimes diarrhea, unconsciousness, and difficulty in breathing. This is much simpler and is perhaps more understandable by the public. I don't think the rest is needed."

RESPONSE: *As stated previously, the text was shortened somewhat and the wording of some effects was simplified.*

COMMENT: P3, L31-33: "Why not BuChE or AChE assays in blood samples to show exposure to any OP or carbamate. If normal then reassuring that tribufos exposure has not occurred recently."

RESPONSE: *Information regarding the use of butyrylcholinesterase and acetylcholinesterase is part of the medical test answer in Chapter 1.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

COMMENT: P7, L11-15: "Not sure what you mean by 'severe' – many patients with relatively modest exposure will feel ill, perhaps vomit, have pinpoint pupils, and be covered with sweat. Severe inhibition is associated with coma and respiratory arrest. This combines all effects of AChE inhibition and calls in severe, which is not accurate." The Reviewer also noted that the majority are not symptoms and suggested changing "symptoms" to "features".

RESPONSE: *The word "severe" was deleted and the suggested wording changes were made.*

COMMENT: P8, L23: "No mention of hen studies showing NTE inhibition."

RESPONSE: *As stated previously, brief discussions of the results from studies of hens administered tribufos were added to Sections 2.2 and 3.2 of the profile, along with the following statement: "Although studies of hens are useful for hazard identification, applicability of the dose-response in hens to humans is uncertain."*

COMMENT: P10, L12: The Reviewer noted that neurotoxic esterase was not previously mentioned in the health effects section of Chapter 2. The Reviewer indicated that this point was made in previous comments.

RESPONSE: *The Reviewer associated this comment with Sections 2.3 and 3.2.1. See previous response.*

CHAPTER 3. HEALTH EFFECTS

COMMENT: P17, L16: "Why no mention of NTE inhibition and development of OPIDN? See http://www.atsdr.cdc.gov/csem/csem.asp?csem=11&po=29 Although this entry needs updating markedly. Some of the references have been discredited and the enzyme has now been cloned."

RESPONSE: *As stated previously, brief discussions of the results from studies of hens administered tribufos were added to Sections 2.2 and 3.2.1 of the profile, along with the following statement: "Although studies of hens are useful for hazard identification, applicability of the dose-response in hens to humans is uncertain."*

COMMENT: P21, L15-18: "First mention of this issue. Accept difficulty of relating dose response from chickens to humans, but surprised that there is no mention of the studies showing OPIDN to occur with >70% inhibition of NTE."

RESPONSE: *See previous response.*

COMMENT: P26, L8: Regarding the statement "Therefore, hen/chicken studies are not included in this section", the Reviewer indicated that the study results from hens/chickens "should be included somewhere as relevant to humans."

RESPONSE: *As stated previously, brief discussions of the results from studies of hens administered tribufos were added to Sections 2.2 and 3.2 of the profile, along with the following statement: "Although*

studies of hens are useful for hazard identification, applicability of the dose-response in hens to humans is uncertain."

COMMENT: P35, L31: The Reviewer suggested adding the phrase "intermediate exposure" to the summary of a study of young adult New Zealand white rabbits receiving 6 hour occluded dermal application of tribufos 5 days/week for up to 3 weeks.

RESPONSE: *The suggested addition was not made because some of the animals died within a time period that was acute duration (i.e., <15 days).*

COMMENT: P37, L4: The Reviewer stated that the report of decreased neurotoxic esterase in lymphocytes from workers reportedly exposed to tribufos and folex should be discussed in the pathogenesis section.

RESPONSE: *The finding of decreased NTE in the seven workers is not discussed in Section 3.2 because there were no clinical signs of exposure-related neurotoxicity.*

COMMENT: P38, L4: The Reviewer asked whether duration is needed for exposure scenarios reported in the genotoxicity section of the toxicological profile.

RESPONSE: *Exposure duration data were added when available; otherwise, a statement was added to note that exposure duration information was not presented in available secondary sources.*

COMMENT: P46, L18: The Reviewer stated that NTE inhibition and OPIDN data are ignored in the Mechanisms of Toxicity section of the toxicological profile.

RESPONSE: *Mechanisms of OPIDN are not discussed in Section 3.5.2 (Mechanisms of Toxicity) because tribufos-mediated OPIDN has not been demonstrated in humans or laboratory mammals.*

COMMENT: P48, L31: The Reviewer stated that Section 3.7 (Children's Susceptibility) includes very old literature (e.g., 1960s and 70s) and that there is more recent literature that might be relevant.

RESPONSE: *It appears that the Reviewer refers to boilerplate citations. ATSDR will evaluate the boilerplate for possible update information.*

COMMENT: P52, L17: In reference to Section 3.8.1 (Biomarkers Used to Identify or Quantify Exposure to Tribufos, the Reviewer stated "AChE, BuChE, and lymphocyte NTE activities have been used to record exposure."

RESPONSE: *As stated previously, information regarding the use of butyrylcholinesterase, acetylcholinesterase, and neurotoxic esterase as biomarkers of effect to substances such as tribufos was added to Section 3.8.2.*

COMMENT: P54, L23: Regarding the statement "plasma ChE acts as a depot for tribufos….", the Reviewer stated "I do not think that tribufos can be reformed after ChE inhibition and therefore it is not a depot."

RESPONSE: *The statement in question was deleted in response to a comment by another reviewer of the toxicological profile.*

COMMENT: Section 3.11 (Methods for Reducing Toxic Effects): The Reviewer stated that texts of Erdman (2004) and Osmundson (1998) are old and outdated and that Aaron (2007) is better and more easily obtained. The Reviewer indicated that more recent reviews based on clinical experience include: "Eddleston M, Buckley NA, Eyer P, Dawson AH. Medical management of acute organophosphorus pesticide poisoning. Lancet 2008, 371: 597-607" and "Eddleston M. Insecticides: organic phosphorus compounds and carbamates. In: Goldfrank's Toxicologic Emergencies, 10th edition. Ed: Nelson L et al, McGraw-Hill: New York, 2015."

RESPONSE: *Erdman (2004) and Osmundson (1998) were deleted and Eddleston (2015) and EPA (2013b) were added.*

COMMENT: Section 3.11 (Methods for Reducing Toxic Effects): The Reviewer suggested adding a subsection titled "Resuscitation and stabilization" that states the following: "All patients should be resuscitated and stabilized, with provision of oxygen, intravenous fluids and atropine, intubation and mechanical ventilations, as necessary. Atropine should be given in a doubling dose regimen, titrated to the individu[a]l patient's response to treatment (Abedin 2012)." The Reviewer provided the source of the statement: "Abedin MJ, Sayeed AA, Basher A, Maude RJ, Hoque G, and Faiz MA. (2012). Open-label randomized clinical trial of atropine bolus injection versus incremental boluses plus infusion for organophosphate poisoning in Bangladesh. Journal of Medical Toxicology, 8(2), 108–17."

RESPONSE: *The text in the paragraph immediately preceding Section 3.11.1 (Reducing Peak Absorption Following Exposure) was revised to include the following: "Cases of suspected organophosphorus poisoning should initially be resuscitated (if necessary) and stabilized."*

COMMENT: The Reviewer suggested several revisions to the sections titled Reducing Peak Absorption Following Exposure, Reducing Body Burden, and Interfering with the Mechanism of Action for Toxic Effects. The suggestions included deleting text regarding the use of ethyl alcohol, updating a reference on charcoal effectiveness, and other minor editorial points.

RESPONSE: *Text regarding the use of ethyl alcohol was deleted. The reference regarding the effectiveness of charcoal was updated. Minor editorial comments were incorporated where deemed appropriate.*

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

COMMENT: Table 5-2: The Reviewer stated that for the source of information in Table 5-2 (NPIRS 2016), the abbreviation NPIRS is not included in the abbreviation list.

RESPONSE: *NPIRS is a reference; the abbreviation stands for National Pesticide Information Retrieval System and is so identified in Chapter 9 (References).*

CHAPTER 7. ANALYTICAL METHODS

COMMENT: Section 7.3.1 (Identification of Data Needs) under Methods for Determining Biomarkers of Exposure and Effect: The Reviewer again mentioned that neurotoxic esterase is mentioned in the section titled "Exposure", but that no context is given in earlier sections. The Reviewer also stated "AChE and BuChE can be used as biomarkers of OP or carbamate exposure."

RESPONSE: *Information regarding biomarkers AChE and BuChE was added to this section.*

COMMENT: Section 7.3.1 (Identification of Data Needs) under Methods for Determining Biomarkers of Exposure and Effect: In the section titled "Effect", the Reviewer suggested that the statement "Tribufos affects the central nervous system by inhibiting ChE" should be clarified to note that it inhibits "neural AChE".

RESPONSE: *ChE was replaced with AChE.*

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

COMMENT: Table A-1: The Reviewer stated "Need to control for red cell haematocrit since variation (e.g., anaemia) will result in variation. Should be mentioned in the earlier discussions of AChE as biomarker. Clearly has not been done in this study, so cannot be added."

RESPONSE: *The unpublished (and therefore, uncitable) study report included red blood cell, hemoglobin, and hematocrit data from exposure weeks 4, 8, and 13. It was clear that exposure had no effect on these parameters, with the possible exception of male rats of the highest exposure level. The PHS statement (ARE THERE MEDICAL TESTS TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO TRIBUFOS?) was revised to include the following statement: "Your doctor may also need to check your red blood cell and hemoglobin levels because low levels of these blood elements could cause lower than normal activity of AChE in your blood."*

Comments provided by Reviewer #2:

General Comment

The Reviewer stated "This Toxicological Profile of tribufos is well written and generally well documented. Most of my concerns and comments are minor in nature. I have identified three major issues that are discussed in detail in the following section of this review:

- Exposure of children of agricultural workers
- Exposure and health effects of children living near treated fields
- Pesticide drift into communities near treated fields"

RESPONSE: *No response is necessary; the three major issues raised are discussed in detail elsewhere in the Reviewers comments.*

Comments on Charge Questions and Statements from the Guidelines for Peer Review of ATSDR's Toxicological Profiles

Charge question and statement: "Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?"

COMMENT: "Yes. There have been several studies of organophosphorus pesticide exposure and children's health that are not discussed. Please see Major Issues #2 below."

RESPONSE: *See response to Major Issue #2 below.*

Charge question and statement: "Are there any general issues relevant to child health that have not been discussed in the profile and should be?"

COMMENT: "The issue of the exposure of children of agricultural workers has been discussed, but could be improved. Please see Major Issues #1 below."

RESPONSE: *See response to Major Issue #1 below.*

CHAPTER 1. PUBLIC HEALTH STATEMENT

Charge question and statement: "The tone of the chapter should be factual rather than judgmental. Does the chapter present the important information in a non-technical style suitable for the average citizen? If not, suggest alternate wording."

COMMENT: "Yes."

RESPONSE: *No response is necessary.*

Charge question and statement: "Major headings are stated as a question. In your opinion, do the answers to the questions adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them."

COMMENT: "Yes, for the most part. Please see comments in the text and the Major Issues section."

RESPONSE: *See responses to comments on annotated pages and the major issues section below.*

Charge question and statement: "Are scientific terms used that are too technical or that require additional explanation? Please note such terms and suggest alternate wording."

COMMENT: "The terms used in this chapter seem appropriate for the audience."

RESPONSE: *No response is necessary.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

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

COMMENT: "Yes."

RESPONSE: *No response is necessary.*

Charge question and statement: "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: "In the absence of information on human health effects, animal studies are helpful in understanding potential health effects in humans."

RESPONSE: *No response is necessary.*

Charge question and statement: "Have exposure conditions been adequately described? If you do not agree, please explain."

COMMENT: "Several concerns are raised in the Major Issues section of this review."

RESPONSE: *See responses to concerns in the major issues section of the Reviewer #2 review.*

CHAPTER 3. HEALTH EFFECTS

Charge question and statement: "The intended audience for this chapter includes community-level public health officials, physicians, and concerned citizens. It is not intended to be a data review for toxicologists. Emphasis is placed on providing a summary evaluation of the weight of evidence, rather than on providing detailed descriptions of every relevant study. Scientifically prudent judgments and interpretations are both appropriate and desirable."

COMMENT: "Very little information on human exposure and health effects in humans was available for tribufos. The information that was available was reviewed adequately.

Animal studies were reviewed carefully and exhaustively. I am not sure that the level of detail for the individual animal toxicity studies contained in this profile is really of interest to community-level public health officials, physicians, and concerned citizens. As an example, Section 3.2.1.4 discusses Neurological Effects. The discussion of animal studies includes specification of the mass median aerodynamic diameter range for particles used in nose-only aerosol studies. Is this type of information truly of interest to the audiences you identify?

Levels of Significant Exposure (LSE) Tables and Figures were clear and helpful."

RESPONSE: *The detailed information is considered useful to some readers.*

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

Charge question and statement: "Is information provided on the various forms of the substance? If not, please explain."

COMMENT: "The information provided appears accurate and adequate."

RESPONSE: *No response is necessary.*

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

Charge question and statement: "Are you aware of any information that is wrong or missing? If so, please provide copies of the references and indicate where (in the text) the references should be included."

COMMENT: "No."

RESPONSE: *No response is necessary.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

Charge question and statement: "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 does not fully address the issue of pesticide drift. Please see Major Issue #3."

RESPONSE: *See response to Major Issue #3 below.*

Charge question and statement: "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: "Some of the information provided regarding tribufos volatilization and behavior in the atmosphere was not clear. Please see Major Issue #3."

RESPONSE: *See response to Major Issue #3 below.*

Charge question and statement: "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: "Yes, for the most part. Please see Major Issue #3."

RESPONSE: *See response to Major Issue #3 below.*

Charge question and statement: "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: "The profile describes well the dietary exposure pathway and the ingestion route for food and water. It also provides an appropriate focus on occupational exposures. The profile would be improved by a more thorough discussion of exposure children of agricultural workers, and of people living near treated fields; particularly, children living near treated fields. Please the Major Issues section below."

RESPONSE: *See responses to major issues below.*

CHAPTER 7. ANALYTICAL METHODS

This chapter begins with standard language (in bold). Most information should be presented in tabular form.

Charge question and statement: "Are you aware of additional methods that can be added to the tables? If so, please provide copies of appropriate references."

COMMENT: "No."

RESPONSE: *No response is necessary.*

Charge question and statement: "Have methods been included for measuring key metabolites mentioned previously in the text?"

COMMENT: "These have been mentioned in the text."

RESPONSE: *No response is necessary.*

Charge question and statement: "If unique issues related to sampling for the substance exist, have they been adequately addressed in the text? What other discussion should be provided?"

COMMENT: "N/A."

RESPONSE: *No response is necessary.*

CHAPTER 8. REGULATIONS AND ADVISORIES

Charge question and statement: "Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference."

COMMENT: "No."

RESPONSE: *No response is necessary.*

CHAPTER 9. REFERENCES

Charge question and statement: "Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional reference."

COMMENT: "Additional references have been provided for the authors' consideration in the Major Issues section below."

RESPONSE: *See responses to major issues below.*

Major Issues Presented by Reviewer #2

Issue 1: Exposures of children of agricultural workers

COMMENT: The following comment pertains to Section 6.6 (Exposures of Children) and Section 6.8.1 (Identification of Data Needs; Exposures of Children): "Over the past two decades several research groups have documented what is sometimes referred to as the pesticide take home pathway for agricultural workers. There are now clear recommendations for workers regarding work clothing." "It would be very helpful to have a short description of this issue in the profile, along with a more strongly stated recommendation for workers to take off work clothes when returning home, and for washing work clothes separate from family clothes." The Reviewer provided background information and a list of 10 references that are considered relevant to the issue.

RESPONSE: *ATSDR has added information to Chapter 1 (How Can Families Reduce the Risk of Exposure to Tribufos) and Chapter 6.7 (Populations with Potentially High Exposures), which address the Reviewer's comments. None of the above cited articles specifically address tribufos; however, ATSDR has cited Curl et al. (2002), Loewenherz et al. (1997), and Lu et al. (2000) to convey that good hygiene practices (change work clothes before entering the home and wash work clothes separately from other family clothing) can reduce exposure to children whose parents may work with tribufos.*

References considered pertinent to Issue 1:

Bradman MA, Harnly ME, Draper W, Seidel S, Teran S, Wakeham D, Neutra R. 1997. Pesticide exposures to children from California's Central Valley: results of a pilot study. J Expo Anal Environ Epidemiol. 7(2):217-34.

Curl CL, Fenske RA, Kissel JC, Shirai, JH, Moate TF, Griffith W, Coronado G, Thompson B. 2002. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. Environ Health Perspect. 110(12):A787-92.

Fenske RA, Lu C, Negrete M, Galvin K. 2013. Breaking the take home pesticide exposure pathway for agricultural families: workplace predictors of residential contamination. Am J Ind Med. 56(9):1063-71.

Loewenherz C, Fenske RA, Simcox NJ, Bellamy G, Kalman D. 1997. Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington State. Environ Health Perspect. 105(12):1344-53.

Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res. 84(3):290-302.

McCauley LA, Michaels S, Rothlein J, Muniz J, Lasarev M, Ebbert C. 2003. Pesticide exposure and self reported home hygiene: practices in agricultural families. AAOHN J. 51(3):113-9.

McCauley LA, Travers R, Lasarev M, Muniz J, Nailon R. 2006. Effectiveness of cleaning practices in removing pesticides from home environments. J Agromedicine. 11(2):81-8.

Salvatore AL, Castorina R, Camacho J, Morga N, López J, Nishioka M, Barr DB, Eskenazi B, Bradman A. 2015. Home-based community health worker intervention to reduce pesticide exposures to farmworkers' children: a randomized-controlled trial. J Expo Sci Environ Epidemiol. 25(6):608-15.

Sandel M, Baeder A, Bradman A, Hughes J, Mitchell C, Shaughnessy R, Takaro TK, Jacobs DE. 2010. Housing interventions and control of health-related chemical agents: a review of the evidence. J Public Health Manag Pract. 16(5 Suppl):S24-33.

Simcox NJ, Fenske RA, Wolz SA, Lee IC, Kalman DA. 1995. Pesticides in household dust and soil: exposure pathways for children of agricultural families. Environ Health Perspect. 103(12):1126-34.

Issue 2: Exposure and health effects among children living near treated fields

COMMENT: The following comment pertains to Section 3.7 (Children's Susceptibility), Section 6.6 (Exposures of Children) and Section 6.8.1 (Identification of Data Needs; Exposures of Children): "Two major epidemiologic studies have evaluated organophosphate exposures among children living in agricultural communities. Both studies found elevated risks for specific health outcomes and OP pesticide exposure. Although these studies did not focus on tribufos, it would be informative for your readers to know that these associations have been observed for the same class of pesticides." "It would be helpful to include a short description of the findings of the CHAMACOS and CHARGE studies (see below) as they pertain to OP pesticides." The Reviewer provided background information and a list of 11 references that are considered relevant to the issue.

RESPONSE: *Section 3.7 was revised to indicate that age-related differences in susceptibility have been noted for other OPs, but not for tribufos.*

References considered pertinent to Issue 2:

CERCH. 2016. Center for Environmental Research and Children's Health. Accessed Mar 6 2016 at http://cerch.org/research-programs/chamacos/

Bouchard MF, Chevrier J, Harley KG, et al. 2011. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect 119(8):1189–95.

Engel SM, Wetmur J, Chen J, et al. 2011. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect 119(8):1182–8.

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect 115(5):792-8.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 112(10):1116-24.

Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. 2010. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ Health Perspect 118(12):1768-74.

NIEHS/EPA. 2016. Children's Environmental Health and Disease Prevention Research Centers. National Institute of Environmental Health Sciences and U.S. Environmental Protection Agency. Accessed Mar 6, 2016 at http://www.niehs.nih.gov/research/supported/centers/prevention/

Rauh VA, Garfinkel R, Perera FP, et al. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118(6):e1845-59.

Rauh V, Arunajadai S, Horton M, et al. 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect 119(8):1196–1201

Shelton JF, Geraghty EM, Trancrdi DJ et al. 2014. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE Study. Environ Health Perspect 122(10):1103-09.

Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, Barr DB, Furlong CE, Holland NT. 2005. Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. Neurotoxicology 26(2):199-209.

Issue 3: Pesticide drift and communities near treated fields

COMMENT: The following comment pertains to Section 2.1 (Background and Environmental Exposures to S,S,S-Tributyl Phosphorotrithioate [Tribufos} in the United States) and Chapter 6 (Potential for Human Exposure): "The profile provides a mixed message regarding pesticide drift and potential exposures for those living near treated fields. On the one hand, the profile mentions several times that exposure might occur in areas near treated fields, and suggests that nearby residents might want to stay indoors during spraying. In other parts of the document, however, the movement of tribufos in air following applications is treated as a minor or negligible concern. Overall, the profile conveys the general impression that airborne tribufos following treatment is of little public health concern." "It would be helpful for the authors to carefully review their discussion of tribufos in air, making sure that statements in the profile are supported by the scientific articles cited. They may also wish to consider the information provided below in Background Information." The Reviewer provided background information and a list of 5 references that they found relevant to the issue. The Reviewer further stated the following: "Nevertheless, the profile conveys the general impression that airborne tribufos following treatment is of little public health concern. I recommend that the authors review and perhaps revise the following statements related to tribufos in air:

- "A statement that tribufos is not involved in long-range atmospheric transport is made at least three times in the profile, but no references are cited to substantiate this statement. Section 1

(Public Health Statement, p.1) states, "Tribufos does not travel long distances in air". I can find no evidence in the profile to support this statement."

RESPONSE: *The Reviewer is confusing long-range atmospheric transport (LRT) with spray drift. LRT occurs for substances that are extremely slow to degrade in the atmosphere and are typically found in monitoring studies in remote locations (e.g., Arctic) where there are no possible local sources. Volatile pollutants with atmospheric lifetimes of years or longer are subject to LRT. Some examples of such substances are chlorofluorocarbons, perfluorocarbons, and halogenated solvents like perchloroethylene. Tribufos has a short atmospheric half-life (Meylan and Howard 1993 and EPA 2012h) and has never been detected in air samples several hundred kilometers from where it was applied; thus, it is not subject to LRT and no references will exist discussing LRT of tribufos since it is not detected in remote locations. Spray drift refers to the short distance (a few kilometers or so) atmospheric transport immediately following application. The profile provides several examples where tribufos was detected outside the target area following aerial application. These are examples of spray drift. ATSDR agrees that populations near fields treated with tribufos may be exposed via spray drift. ATSDR has concluded that the vast majority of U.S. residents are not exposed to tribufos through LRT. Populations in states such as New York, New Jersey, Pennsylvania, Minnesota, Wisconsin, Michigan, Oregon, etc. will have no exposure to tribufos following application to a cotton field located in Georgia, Texas, Mississippi, Alabama, and so forth because the potential for LRT of tribufos is low.*

- "A half-life value of "approximately two hours" for tribufos in air is reported in the profile on page 6, citing Meylan and Howard 1993. The same statement is made on p.68 with no reference cited. But, unless I am mistaken, the Meylan and Howard article does not mention tribufos. I did not see tribufos listed in any of the tables in this article. Am I missing something? Also, the article does not report half-life data; instead, it reports rate constants using two different models. If a half-life of two hours is reported in this article, I couldn't find it."

RESPONSE: *The paper cited by the Reviewer does not quote a half-life of tribufos per se. It discusses the structure estimation method that is used to estimate the second-order hydroxyl radical rate constant. This can be done by hand for tribufos (or other chemicals) using the data from the paper, but is more commonly performed using a computer program such as the EPA EPIWIN software. The half-life is obtained by multiplying the rate constant by an assumed hydroxyl radical concentration. For example, the hydroxyl radical rate constant for tribufos was calculated as* 7.9×10^{-11} cm^3*/molecule-second. The pseudo first-order half-life is:*

$$t_{1/2} = 0.693/7.9 \times 10^{-11}\ cm^3/\text{molecule-second} \times 1.5 \times 106\ \text{molecules}/cm^3 = 5{,}848\ \text{seconds} \sim 1.63\ \text{hours}.$$

This calculation is explained in Section 6.3.2.1. ATSDR will consider changing the reference to point to the computer modelling software (EPIWIN), which actually calculated the half-life using the method in the paper discussed above.

- "The Potter et al. 2002 article regarding soil dissipation of tribufos provided fairly strong evidence that a substantial fraction of tribufos in soil is escaping into the atmosphere. The Potter article indicates that volatilization is an important dissipation pathway for tribufos in soil: "the data suggest that volatilization was a primary pathway". Tribufos has a relatively low vapor pressure, but it is still considered a semi-volatile compound. Potter et al. refer to tribufos as among a group of 'moderately volatile pesticides'."

RESPONSE: *The Registration Eligibility Decision (RED) document from EPA (EPA 2006b) as well as the risk assessment document for tribufos use to federally threatened California red-legged frog (EPA 2008) state that potential for volatilization from soil and water is expected to be low. The Potter et al. (2002) conclusion that*

volatilization was a primary removal pathway is based on the observation that tribufos dissipated more rapidly in field studies where volatilization was possible versus laboratory studies in which the test vessels were sealed. The assumption is that the degradation lifetimes should be similar in both the laboratory and field studies and there was little loss of tribufos through runoff in the field studies; thus, the only other explanation for the enhanced loss in field studies was volatilization. ATSDR agrees there is uncertainty regarding the degree to which tribufos will volatilize from treated cotton fields; ATSDR states that there is a data need for more research (field volatility study) in this area. Even though there may be some volatilization, LRT is not occurring because tribufos is broken down by hydroxyl radicals. Hermann and Seiber 1981 and Kilgore et al. 1984 showed that atmospheric levels decrease very quickly with time in areas where applied. Only 38% remained at 24 hours post application and 2% at 3 days. Even at the levels recorded in drift studies (high of 1189 ng/m3), data indicates that the exposure is 2.5 to 10 below the MRL (level of concern) so health effects are unlikely.

It seems clear from all of the available scientific studies that tribufos is moderately volatile, that volatilization is a primary pathway for soil dissipation, and that most air monitoring studies that have looked for tribufos have found it. If these statements are correct, then I suggest that the profile be revised accordingly."

RESPONSE: *See responses to comments above.*

References considered pertinent to Issue 3:

Gibbs JL, Yost MG, Negrete M, Fenske RA. 2016. Passive sampling for indoor and outdoor exposures of chlorpyrifos, azinphos-methyl, and oxygen analogs in a rural agricultural community. Environ Health Perspect. 2016 Aug 12. [Epub ahead of print] PMID: 27517732.

Harnly, M; McLaughlin, R; Bradman, A; Anderson, M; and Gunier, R. Correlating agricultural use of organophosphates with outdoor air concentrations: a particular concern for children. 2005. Environmental Health Perspectives. 113(9), 1184-1189.

Popendorf W, Leffingwell T. 1978. Natural variations in the decay and oxidation of parathion foliar residues. J Agric Food Chem. 26:437.

Spear RC, Lee Y, Leffingwell T, Jenkins D. 1978. Conversion of parathion to paraoxon in foliar residues: effects of dust level and ozone concentration. J. Agric. Food Chemistry. 26: 434-436.

Van den Berg F, Kubiak R, Benjey WG, Majewski MS, Yates SR, Reeves GL, Smelt J, Van Der Linden AMA. 1999. Emission of Pesticides into the Air. Water Air Soil Pollut. 115:195.

Specific Comments on Annotated Pages of the Toxicological Profile for Tribufos

CHAPTER 1. PUBLIC HEALTH STATEMENT

COMMENT: P1, L24 and other locations: "It is not clear why you are citing references in this section. My impression is that you want this public health statement to be reader-friendly. I would probably omit the few reference citations; alternatively, if you want to make such citations, then you should be consistent and do so for most of the information in the section."

RESPONSE: *References are removed from Chapter 1 of public-comment and final drafts of toxicological profiles.*

COMMENT: P1, L32 (What Happens to Tribufos when it Enters the Environment?): Regarding the statement "Tribufos does not travel long distances in air", the Reviewer stated: "I am not able to find documentation of this statement in the profile."

RESPONSE: *Substances subject to long range transport have long atmospheric persistence times and are detected in remote regions (e.g., Arctic) where there is no potential for local releases. This is not true for tribufos.*

COMMENT: P3, L10-11 (How Can Tribufos Affect Children?): Regarding the statement "We do not know whether children would be more sensitive than adults to tribufos", the Reviewer stated: "On Page 8, line 9, it is stated: 'Results from acute-duration oral studies in rats indicate that neonates may be more sensitive than adults to tribufos neurotoxicity.' There is no acknowledgment of these findings, whereas this paragraph goes on to cite other animal studies as evidence of effects. It is clear that we do not know much about tribufos effects in humans. If animal studies are going to be invoked here, then I suggest doing so in terms of increased sensitivity in neonates."

RESPONSE: *This section was revised to state the following: "We do not know whether children would be more sensitive than adults to tribufos toxicity. We do not know whether exposure to tribufos might cause birth defects or other developmental effects in people. In animals, levels of exposure to tribufos high enough to affect the health of pregnant mothers caused decreased numbers of rats born and decreased survival. These exposure levels were many times higher than levels allowed in human food sources."*

COMMENT: P3, L14-15 (How Can Tribufos Affect Children?): Regarding the statement "These exposure levels were many times higher than levels allowed in human food sources", the Reviewer stated: "This statement is correct, but does not consider occupational exposures. Can the same be said for pregnant mothers who handle tribufos or who work in recently treated fields?"

RESPONSE: *No revision necessary. It is generally understood that workers who apply pesticides and workers in recently treated areas are at greater risk of significant exposure.*

COMMENT: P3, L25-26 (How Can Families Reduce the Risk of Exposure to Tribufos?): Regarding the statement "Agricultural workers who come into contact with tribufos may want to remove and wash clothing and shower before joining other family members", the Reviewer stated: "I would change this language to 'should always'. Many research and education groups have been stressing the importance of reducing what is referred to sometimes as 'take home' exposure. Workers should not feel that these steps to protect children are optional. Please see my summary comments."

RESPONSE: *ATSDR revised to use the words "should consider."*

COMMENT: P3, L24 (What Recommendations has the Federal Government Made to Protect Human Health?): Regarding the use of the term "leftovers", the Reviewer stated: "I have never seen this term used to describe chemicals that remain on crops. It seems awkward. I would omit or find another term, such as 'chemicals remaining on crops at harvest'."

RESPONSE: *The term "leftovers" was replaced with the following: "(tribufos and/or its breakdown products that stick to food or crops eaten by humans or animals)."*

COMMENT: P3, L29 (What Recommendations has the Federal Government Made to Protect Human Health?): Regarding the statement "For more information on regulations and advisories, see Chapter 8", the Reviewer stated: "There is no information here on occupational exposures. Perhaps the authors feel that mention of worker exposure here is not appropriate, as this document is aimed the general public. But we have many health care providers who have agricultural workers among their clients in rural WA State. Presumably, this is true in cotton growing regions throughout the U.S."

RESPONSE: *No revision necessary. Approved application rates and re-entry intervals are provided in Chapter 5. Agricultural worker recommendations are not government regulations; thus, they do not appear in Chapter 8.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

COMMENT: P6, L14 (Background and Environmental Exposures to S,S,S-Tributyl Phosphorotrithioate (Tribufos) in the United States): Regarding the statement that the estimated atmospheric half-life of tribufos is approximately 2 hours, the Reviewer stated: "I did not see tribufos listed in any of the tables in this article. Am I missing something? Also, the article does not report half-life data; instead, it reports rate constants using two different models. If a half-life of two hours is reported in this article, I couldn't find it. Nearly all air monitoring studies conducted near cotton fields that are cited later in this document found measurable amounts of tribufos, albeit in low concentrations. The Potter article here provided fairly strong evidence that a substantial fraction of tribufos in soil is escaping into the atmosphere. Tribufos does have a relatively low vapor pressure, but it is still considered a semi-volatile compound. My point is that exposure near treated fields should not be downplayed too much. Please see my summary comments."

RESPONSE: *No revision necessary. The reference is for the estimation method to derive rate constants for the reaction of a substance with hydroxyl radicals that computer programs use to estimate an atmospheric half-life. Please also refer to the response for major issue 3.*

COMMENT: P6, L16-17 (Background and Environmental Exposures to S,S,S-Tributyl Phosphorotrithioate (Tribufos) in the United States): Regarding the statement "volatilization from soils under hot and humid conditions may occur", the Reviewer stated "The Potter article seems to indicate that volatilization is an important dissipation pathway; saying 'may occur' seems to misrepresent the article's findings. Potter refers to tribufos as among a group of 'moderately volatile pesticides'."

RESPONSE: *The text was revised to state the following: "…volatilization from water and soil surfaces is expected to occur slowly; however, a field dissipation study indicated that volatilization from soils under hot and humid conditions may be an important environmental fate process."*

COMMENT: P6, L28 (Background and Environmental Exposures to S,S,S-Tributyl Phosphorotrithioate (Tribufos) in the United States): Regarding the statement "Exposure to tribufos to the general population is extremely low", the Reviewer stated "Agree. It is good to see this stated so clearly."

RESPONSE: *No response is necessary.*

COMMENT: P6, L31-32 (Background and Environmental Exposures to S,S,S-Tributyl Phosphorotrithioate (Tribufos) in the United States): Regarding the statement "Inhalation exposure to tribufos is expected to be negligible for the general population…", the Reviewer stated "Agree again."

RESPONSE: *No response is necessary.*

COMMENT: P6, L33-34 (Background and Environmental Exposures to S,S,S-Tributyl Phosphorotrithioate (Tribufos) in the United States): Regarding the statement "Since tribufos is rarely detected in groundwater or drinking water, this is not considered an important exposure pathway for the general population", the Reviewer stated "Agree."

RESPONSE: *No response is necessary.*

COMMENT: P7, L1-3 (Section 2.2 Summary of Health Effects): Regarding the statement "The absorbed daily dose of workers during and following application was estimated to range from about 1 to 25 µg/kg/day depending upon job function (see Section 6.5)", the Reviewer stated "In stating the estimated doses from dietary intake earlier in the paragraph the authors cite EPA 2006b. Here there is a statement of estimated doses for workers, but no citation. I suggest you follow a consistent approach. Either cite in both cases, or refer the reader to a later section in both cases."

RESPONSE: *Revisions in this area precluded the need to revise with the source.*

COMMENT: P7, L20-28 (Section 2.2 Summary of Health Effects): Regarding discussion of degree of AChE inhibition and its designation as a "less serious effect" or "serious effect", the Reviewer stated "I was not familiar with the 'serious' and 'less serious' categories that ATSDR has developed before I read this profile. I looked at the article cited (Chou and Williams-Johnson 1998) and found it helpful. But I don't believe that this terminology is used by any other risk evaluation agency. If that is the case, then I think it would be worthwhile providing a more complete explanation of how and why ATSDR uses this system. Does this categorization apply only to neurological effects? What is meant by 'significant dysfunction'?"

RESPONSE: *No revision necessary. An explanation of the terms "less serious effect" and "serious effect" is provided in the boilerplate text of Section 3.2 of all toxicological profiles. The term "significant dysfunction" refers to a treatment-related impairment that threatens survival. It requires some degree of professional judgment.*

CHAPTER 3. HEALTH EFFECTS

COMMENT: P15, L22 (Section 3.2 Discussion of Health Effects by Route of Exposure): Regarding the term "levels of significant exposure", the Reviewer stated "This term has not been defined. I am not sure what it means. Is a NOAEL a "level of significant exposure"? In Figure 3-1, for example, most of the points are NOAELs. It would be helpful for the authors to explain what they mean here, as this language is not used by EPA or other regulatory agencies."

RESPONSE: *The boilerplate text in Section 3.2 describes levels of significant exposure in terms of NOAELs (no-observed-adverse-effect levels) and LOAELs (lowest-observed-adverse-effect levels) which are subcategorized as "less serious effects" or "serious effects."*

COMMENT: P15, L22 (Section 3.2 Discussion of Health Effects by Route of Exposure): Regarding the sentence "Levels of significant exposure for each route and duration are presented in tables and illustrated in figures", the Reviewer stated "I am suggesting edits to specify the tables and figures. Figure 3-2 should identify each of the three figures it contains by some notation; here I am suggesting a,b,c."

RESPONSE: *Each section of the LSE tables and figures is identified by duration of exposure (i.e., acute, intermediate, chronic). Additional identification does not appear necessary.*

COMMENT: P15, L22 (Section 3.2 Discussion of Health Effects by Route of Exposure): Regarding the sentence "Levels of significant exposure for each route and duration are presented in tables and illustrated in figures", the Reviewer stated "I would drop the parenthetical phrase, or change it to "levels of exposure used in animal dosing studies". There is a danger here for the reader to equate dose and exposure, which are not the same thing. This profile goes into great detail later to talk about both dose and exposure. I noticed that neither 'exposure' or 'dose' are included in the document's Glossary. I recommend adding them, using either current EPA definitions, or the definitions published by the International Programme on Chemical Safety (IPCS)."

RESPONSE: *The statement in question was revised to read "…the actual doses or exposure levels…". Dose and exposure have been added to the glossary.*

COMMENT: P15, L25 to P16, L4 (Section 3.2 Discussion of Health Effects by Route of Exposure): The Reviewer stated "Here is a nice discussion of the 'serious' and 'less serious' categorization. Perhaps some of this information could be included in Section 2. There is no citation regarding ATSDR "guidelines and policies" that are used to classify end points. Can this be provided? Hopefully it is something that is accessible on the ATSDR website."

RESPONSE: *The terms found in the boilerplate portion of Section 3.2 are defined as presented. ATSDR Guidelines can be found on the web at: https://www.atsdr.cdc.gov/ToxProfiles/index.asp#profiledevelopment. A web hyperlink was added into the toxicological profile text.*

COMMENT: P17, L25 (Section 3.2.1 Inhalation Exposure): The Reviewer stated that MMAD "Needs to be defined the first time it is used."

RESPONSE: *MMAD is defined in Chapter 2 in the first instance where the abbreviation appears.*

COMMENT: P19, L3-4 (Section 3.2.1.2 Systemic Effects, Respiratory): Regarding the statement "A major limitation of this study was the lack of accounting for other possible airborne contaminants …", the Reviewer suggested revising the statement to read "Limitations of this study include the lack of quantitative tribufos exposure data and the lack of accounting for other possible airborne contaminants…" in order to be consistent with limitations stated in the previous paragraph.

RESPONSE: *The suggested revision was made.*

COMMENT: P49, L1-2 (Section 3.7 Children's Susceptibility): Regarding the statement "No information was located regarding potential age-related differences in susceptibility to tribufos toxicity in humans", the Reviewer stated "This statement is correct. However, there have been several epidemiologic studies that have focused on organophosphate exposure during pregnancy, and these studies have shown adverse effects in the children born from those pregnancies. I recommend that a brief discussion of these studies be included here, making clear that the studies quantified exposure to organophosphates other than tribufos. It would seem to be a disservice to the reader to omit any mention of these important new findings that may be relevant to exposure to tribufos. This point is discussed in more detail in my summary comments, and appropriate references are provided there."

RESPONSE: *Although tribufos has been placed in the organophosphate category of pesticides, it does not appear to elicit all of the effects of other organophosphates; as such, it may be misleading to discuss age-related susceptibility. ATSDR found no convincing evidence to suggest age-related differences in tribufos susceptibility. The statement was revised to note that age-related susceptibility data are available for other OPs, but not for tribufos.*

COMMENT: P50, L19 (Section 3.8.1 Biomarkers Used to Identify or Quantify Exposure to Tribufos**)**: Regarding the statement "No information was located regarding biomarkers of tribufos exposure in humans", the Reviewer stated "This statement seems to contradict an earlier statement (Section 2, page 3) that references Kilgore et al. 1984."

RESPONSE: *The statement was replaced by the following: "A biomarker of exposure would be the presence of tribufos in blood or urine."*

COMMENT: P53, L2-3 (Section 3.11 Methods for Reducing Toxic Effects): Regarding the statement "The following texts provide specific information about treatment following exposures to tribufos", the Reviewer stated "I am surprised that the EPA document, "Recognition and Management of Pesticide Poisonings is not cited here (EPA 735K13001; Sixth Edition, 2013)."

RESPONSE: *The referenced EPA document was added to the list of documents that serve as sources of information in Sections 3.11.1, 3.11.2, and 3.11.3.*

COMMENT: P59, L1-2 (Section 3.12.2 Identification of Data Needs, Neurotoxicity): Regarding the statement "No human data were located regarding neurological effects associated with tribufos exposure", the Reviewer stated "This statement seems to contradict an earlier citation of Lotti et al. 1983 (Section 3.2.3.4, page 35)."

RESPONSE: *The statement regarding no human data was deleted. The results of Lotti et al. (1983) were summarized in this section.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

COMMENT: P68, L16-19 (Section 6.1 Overview): Regarding the statements "Vapor-phase tribufos degrades fairly rapidly in the atmosphere with a half-life of approximately 2 hours. Particulate-phase tribufos is removed from the atmosphere by wet and dry deposition. Tribufos adsorbs strongly to soil

surfaces and has low potential to leach into groundwater. The Henrys Law constant for tribufos suggests that volatilization from soil and water surfaces occurs slowly…", the Reviewer stated "The basis for these statements is not clear, unless the Potter et al. 2002 article supports them. But my impression is that the Potter article is a report of the field dissipation study."

RESPONSE: *The citation EPA (2008) was added to the text.*

COMMENT: P70, L5 (Section 6.1 Overview): Regarding the statement that tribufos "does not possess long range transport potential", the Reviewer stated "This statement was used earlier in the document. In both cases, there is no citation of a reference."

RESPONSE: *Substances subject to long-range transport have long atmospheric persistence times and are detected in remote regions (e.g., Arctic) where there is no potential for local releases. Tribufos does not possess long atmospheric persistence and has not been detected in remote regions. A citation for this statement does not appear necessary.*

COMMENT: P72, L25-26(Section 6.3 Environmental Fate, 6.3.1 Transport and Partitioning): Regarding the statement "Vapor-phase tribufos is expected to react with photochemically generated hydroxyl radicals, while particulate-phase tribufos may be removed from the atmosphere by wet and dry deposition", the Reviewer stated "No reference is cited for this statement. The Meylan and Howard 1993 reference discusses reactions with hydroxyl radicals, but I don't believe their analysis included tribufos."

RESPONSE: *No revision necessary. All organic compounds react with hydroxyl radicals in the atmosphere. Particulate phase substances are subject to deposition; this is true for any substance, including tribufos.*

COMMENT: P74, L5 (Section 6.3.2 Transformation and Degradation, 6.3.2.1 Air): Regarding the statement "Vapor-phase tribufos in the ambient atmosphere will be degraded by reaction with photochemically generated hydroxyl radicals", the Reviewer stated "Suggest saying 'can be' rather than 'will be'. The model cited uses assumptions that may not be applicable to certain cotton growing regions. Do we know the typical hydroxyl radical concentrations in such regions?"

RESPONSE: *No revision necessary. Tribufos will be degraded in the atmosphere by reaction with hydroxyl radicals, which are ubiquitous in the atmosphere. The global hydroxyl radical concentration is roughly 9.43±1.3 x10^5 radicals/cm^3; Northern Hemisphere 8.98±2.02 x10^5 radicals/cm^3 and Southern Hemisphere 9.93±2.02x10^5 radicals/cm^3. A 12-hour day is used for the calculation since hydroxyl radicals are generated under sunlight conditions. Prinn et al. (2001) serves as the reference for this information.*

COMMENT: P77, L23 (Section 6.4 Levels Monitored or Estimated in the Environment, 6.4.1 Air): Regarding the statement "Since tribufos is used exclusively as a cotton defoliant and is not subject to long-range atmospheric transport…", the Reviewer stated "This is the third time this statement has been made, but none have a reference. It would be helpful to for the authors to define 'long-range atmospheric transport'. I am not convinced that there are field measurements to support the statement."

RESPONSE: *The term "long-range atmospheric transport" was deleted and the statement was revised to the following: "Since tribufos is used exclusively as a cotton defoliant and has a short atmospheric*

half-life, it is usually only detected in ambient air in cotton growing regions where it has been applied." Chemicals subject to long-range transport are (1) volatile or semi volatile, (2) possess long atmospheric half-lives, and (3) detected in remote areas where there are no known sources. None of this is true for tribufos, which has low volatility, an atmospheric half-life of about 2 hours, and has never been detected remotely.

COMMENT: P81, L16-18 (Section 6.5 General Population and Occupational Exposure): Regarding the statement "Cotton defoliation is typically performed in late fall (October–November) in California with most applications administered by airplane (80%) and ground boom spraying (20%) (Lotti et al. 1983)", the Reviewer stated "I am not sure that a 30 year old reference is appropriate to describe the proportion of tribufos applied by airplane and boom spraying in 2016. Is it possible to get more current information from CalEPA? California has an excellent pesticide use reporting system."

RESPONSE: *The statement was replaced with the following: "Exposure to tribufos tends to be seasonal since cotton defoliation is generally performed on mature bolls approximately 10–14 days prior to the anticipated harvest (Barber et al. 2013). Harvest timing of cotton in the United States differs by region, but defoliation is typically performed in fall (September–November); however, the harvest may also extend into December or early January in some states (USDA 2010)."*

COMMENT: P81, L24 (Section 6.5 General Population and Occupational Exposure): Regarding Section 6.6 (Exposures of Children), the Reviewer stated "This section should discuss the potential for exposure of two groups of children. First, children of workers who are exposed to tribufos. There is extensive scientific evidence that agricultural chemicals can move from the workplace to the home. This issue is discussed in more detail in my summary comments. Second, children who live near tribufos-treated fields. These children can be exposed to air and surface residues. This issue is discussed in more detail in my summary comments."

RESPONSE: *The following was added to Section 6.7 (Populations with Potentially High Exposures): "Children of agricultural employees that work with tribufos are potentially exposed to residues from their parent's work clothing. Researchers have studied organophosphate residues in vehicles and homes of agricultural workers in the state of Washington and determined that the transport of pesticides from the workplace to the residence on a worker's clothing or person could lead to exposure to family members (Curl et al. 2002; Loewenherz et al. 1997; Lu et al. 2000). Take-home exposures to family members can be reduced by changing out of work clothes before entering the home, and laundering work clothes separately from other family clothing." However there are very little data with respect to tribufos residues in the homes of workers occupationally exposed.*

COMMENT: P86, L24 (Section 6.8.1 Identification of Data Needs, Exposures of Children): Regarding Section 6.8.1 (Identification of Data Needs; Exposures of Children), the Reviewer stated "This section should discuss exposure of children of workers and children who live near treated fields, as indicated earlier. Please see my summary comments."

RESPONSE: *This section was revised to include the following statement: "Tribufos is very rarely detected in food sources and the estimated intakes are low; however, tribufos levels have not been assessed in milk of lactating mothers and in maternal/fetal cord blood obtained from individuals living near or working in sites where tribufos is sprayed. This information is needed for adequate assessment of the potential for exposure of developing fetuses/infants to tribufos."*

CHAPTER 10. GLOSSARY

COMMENT: P113, L1: Regarding Chapter 10 (Glossary), the Reviewer stated "Suggest adding definitions of 'exposure' and 'dose'."

RESPONSE: *Dose and exposure have been added to the glossary.*

Comments provided by Reviewer #3:

General Comments

The Reviewer stated "Overall, this profile provides a summary of the potential adverse health effects of the organophosphorus pesticide tribufos. Data regarding human health effects of tribufos are very limited; only two studies were identified reporting the effects of tribufos exposure in humans. Therefore, most of the information provided in this profile refers to the adverse effects of tribufos in animals. In addition to reporting data on the pharmacodynamics and pharmacokinetics or tribufos, the authors describe its chemical characteristics and environmental fate.

There is some discussion relevant to the potential toxicity of tribufos in children. However, as discussed in this review, discussion relevant to potential effects of tribufos in children and during development lacks scientific accuracy. For instance, on page 3 the authors state that tribufos would have the same health effects in children and in adults. Yet, there is clinical evidence that clinical manifestations of organophosphorus exposure in children differ from those seen in adults. In addition, an ever growing body of evidence suggests that prenatal exposure to sub-acute levels of organophosphorus pesticides has detrimental effects on cognitive functions in children. These effects have also been observed following exposure of developing animals to organophosphorus pesticides. No study has assessed the potential neurotoxicity of tribufos in developing mammals. Yet, the authors conclude in the profile that there is no need for additional animal studies to evaluate the neurotoxic effects of tribufos. These points are discussed below and were addressed in the revised profile with references to work published in the literature."

RESPONSE: *The issues presented in this general comments summary are addressed where they appear in comments on specific chapters and sections of the toxicological profile for tribufos listed below and/or on annotated pages.*

Specific Comments

CHAPTER 1. PUBLIC HEALTH STATEMENT

General Comment: "The chapter presents relevant information in non-technical language that is suitable for the average citizen. Overall, the summary statements are consistent and well supported by the technical discussion in the document. Answers to most questions adequately address the major issues associated with the potential health effects of tribufos. A few answers have been complemented in the profile, as describe here."

RESPONSE: *No response is necessary.*

HOW MIGHT I BE EXPOSED TO TRIBUFOS?

COMMENT: "The authors can consider the notion that one may also be exposed to tribufos while consuming meat or milk from livestock fed tribufos-containing cottonseed products." The Reviewer suggested revision of this section to include the following: "You may also be exposed to tribufos if you consume meat or milk from livestock fed tribufos-containing cottonseed products." (P2, L7-8 of annotated pages)

RESPONSE: *The suggested addition was made.*

HOW CAN TRIBUFOS AFFECT MY HEALTH?

COMMENT: "It may be more appropriate to consider that most people are not likely to be exposed to levels of tribufos high enough to cause signs and symptoms of acute toxicity. This is more consistent with the fact that we do not know whether long-term exposure to low levels of tribufos would cause harmful effects in people." The Reviewer suggested revising a sentence to read "Most people are not likely to be exposed to levels of tribufos high enough to cause signs and symptoms of acute toxicity." (P2, L23-24 of annotated pages)

RESPONSE: *The suggested change was made.*

HOW CAN TRIBUFOS AFFECT CHILDREN?

COMMENT: "The authors stated that 'It is likely that children would have the same health effects as adults.' This statement can be misleading because clinical studies have reported that signs of acute toxicity induced by organophosphorus pesticides in children and adults are not the same. In general, the classic signs of organophosphorus intoxication that include profuse secretions, bradycardia, muscle fasciculations, and miosis are less common among children than adults, whereas seizures are more frequently observed among children than adults (e.g., Levy-Khademi et al., 2007). It is also important to mention that clinical studies have provided evidence that exposure of the developing brain to levels of organophosphorus pesticides that are not sufficiently high to induce overt signs of acute toxicity has been associated with cognitive impairments and disruption of the structural integrity of the brain in children. The paragraph has been edited accordingly." The Reviewer suggested replacing the statement "It is likely that children would have the same health effects as adults" with the following: "Clinical studies have reported that signs of acute toxicity induced by organophosphorus pesticides such as tribufos in children and adults are not the same. In general, the classic signs of organophosphorus intoxication that include profuse secretions, bradycardia, muscle fasciculations, and miosis are less common among children than adults, whereas seizures are more frequently observed among children than adults (Levy-Khademi et al., 2007). Clinical studies have also provided evidence that the developing brain is more sensitive than the mature brain to the toxic effects of organophosphorus pesticides. Prolonged exposure of the developing brain to levels of organophosphorus pesticides that are not sufficient high to cause overt signs of acute toxicity has been associated with cognitive deficits and disruption of the structural integrity of the brain in children (Horton et al., 2012; Rauh et al., 2012)." (P3, L12-21 of annotated pages).

RESPONSE: *Although tribufos has been placed in the organophosphate category of pesticides, it does not appear to elicit all of the effects of other organophosphate; as such, it may be misleading to discuss age-related susceptibility. ATSDR found no convincing evidence to suggest age-related differences in tribufos susceptibility.*

ARE THERE MEDICAL TESTS TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO TRIBUFOS?

COMMENT: "It may be adequate to add the following: 'If exposure is confirmed and/or suspected, your doctor may also request a test to determine the activity of the enzyme acetylcholinesterase in your blood.' In general, acetylcholinesterase activity in red blood cells and butyrylcholinesterase activity in plasma are used as biomarkers of exposure to organophosphorus pesticides." The Reviewer suggested adding text to read "It is not generally recommended to get tested unless you work with tribufos or live in areas where exposure is likely and are experiencing health effects. If exposure is confirmed and/or suspected, your doctor may also request a test to determine the activity of the enzyme acetylcholinesterase in your blood." (P4, L8-11 of annotated pages)

RESPONSE: *The suggested addition was made along with information regarding testing for BuChE and NTE activities as well.*

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

General Comment: "Although some effects known to occur in humans exposed to organophosphorus pesticides are adequately described in this profile, a few issues need to be addressed with respect to the clinical manifestations of the intoxication. An explanation is provided below, along with references that help clarify the issues."

RESPONSE: *No response is necessary.*

Section 2.2. SUMMARY OF HEALTH EFFECTS

COMMENT: "In the first paragraph of this section, the authors did not describe the primary role of acetylcholinesterase (AChE). They also indicate that muscarinic and nicotinic receptors are confined to the peripheral nervous system, when in reality these receptors are expressed in the central and peripheral nervous systems. The paragraph has been edited accordingly in the profile." The Reviewer suggested revising the paragraph to read "Tribufos is an organophosphorus compound considered to be of moderate toxicity compared to other organophosphates. The primary mechanism that underlies the acute toxicity induced by organophosphorus toxicity compounds is irreversible inhibition of acetylcholinesterase (AChE), the enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine. Accumulation of acetylcholine leads to overactivation of muscarinic and nicotinic receptors in the peripheral and central nervous systems. Overstimulation of muscarinic receptors in target organs results in excessive glandular secretions (salivation, lacrimation, rhinitis), miosis, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia. Overactivation of nicotinic receptors results in tachycardia, muscle fasciculations, cramping, twitching, muscle weakness, and muscle paralysis. Central nervous system toxicity includes respiratory depression, anxiety, insomnia, headache, apathy, drowsiness, dizziness, loss of concentration, confusion, tremors, convulsions, and coma. These effects, which define a syndrome referred to as cholinergic crisis, usually develop within a few minutes to 24 hours after dosing, depending upon the extent and route of exposure." (P7, L7-22 of annotated pages)

RESPONSE: *The suggested revision was made.*

COMMENT: "In the second paragraph, the authors indicate that AChE expressed in red blood cells (RBCs) is identical to AChE expressed in the nervous system. This is not the case. There are multiple molecular forms of AChE, and the isoforms expressed in brain and skeletal muscles are distinct from those expressed in RBCs (Silman and Sussman, 2005)." The Reviewer suggested revising the sentence "In addition to its presence and function in central and peripheral nervous tissue, AChE is also associated with red blood cells (RBCs)" to read "In addition to its presence and function in central and peripheral nervous tissue, AChE is also expressed in red blood cells (RBCs) (Silman and Sussman, 2005)." (P7, L24-25 of annotated pages)

RESPONSE: *A revision was made to the second paragraph precluding the need to make the edit suggested.*

COMMENT: The Reviewer provided information to indicate that the degree of RBC AChE inhibition does not always correlate with the severity of organophosphorus toxicity, especially when people are chronically exposed to organophosphorus compounds. The Reviewer stated "Therefore, it may be prudent to stress that the categorization of organophosphorus toxicity as "serious" vs. "less serious" on the basis of a threshold level of AChE inhibition only applies to acute toxicity. Based on scientific evidence, neurological deficits can develop following long-term exposure to levels of organophosphorus pesticides that are not anticipated to cause substantial RBC AChE inhibition. The second paragraph has been edited accordingly in the profile." (P7, L25 to P8, L19 of annotated pages).

RESPONSE: *The following statement was added to the paragraph: "The designations of less serious and serious effects on AChE activity are applicable to acute responses to acute-, intermediate-, and/or chronic-duration exposures to organophosphorus compounds such as tribufos." The suggested information regarding the degree of AChE inhibition and its correlation with severity of acute signs of organophosphate toxicity was added to the beginning of Section 3.2, which is considered a more appropriate location for this information.*

COMMENT: The Reviewer stated "Although this may be out of the scope of this profile, the following helps explaining why after repeated exposure to organophosphorus compounds, a high degree of inhibition of RBC AChE does not reflect the degree of inhibition of tissue AChE. Recovery of AChE activity between exposures is due to combined reactivation of the inhibited enzyme and synthesis of new enzyme (Mason, 2000). While the half-life of AChE in the brain ranges from 2 to 3 days (Wenthold et al., 1974), synthesis of new RBC AChE is only due to new RBC production and the half-life of RBCs is approximately 120 days (D'Alessandro et al., 2010)."

RESPONSE: *No revision necessary. The statement by the Reviewer is acknowledged by ATSDR, but is outside the scope of the toxicological profile for tribufos.*

COMMENT: The Reviewer stated "Effects that have only been observed in animals are likely to be of concern to humans. However, a number of issues need to be taken into account when extrapolating results from animal models to humans. For instance, while brain growth spurt is a postnatal event in rats and mice, it is predominantly a prenatal event in long-gestation species, including humans, non-human primates, and guinea pigs. In addition, mice and rats tend to be more resistant to the toxicity of organophosphorus compounds than humans, in part because mice and rats have relatively higher levels of circulating carboxylesterases, the enzymes that metabolize organophosphorus compounds. These issues have been addressed in the review published by Pereira and collaborators (2014) and were briefly introduced in the revised profile." (P8, L21-28 of annotated pages)

RESPONSE: *Information regarding issues to be considered in extrapolating results from animals to humans was added to Section 3.5.3 (Animal-to-Human Extrapolations).*

COMMENT: The Reviewer stated "it is important to define the exposure conditions (sex of the animals, age at which the animals are exposed, dose of tribufos, and duration of exposure)." On the annotated pages, the Reviewer noted multiple locations where additional study details were requested. (P9, L19 to P10, L6 of annotated pages)

RESPONSE: *No revision necessary. Section 2.2 of toxicological profiles is intended to be a brief, high-level summary of health effects. The requested additional study details are not considered a necessary part of this summary. More detailed information is found in Chapter 3, Health Effects.*

COMMENT: The Reviewer stated "the statements that appear in lines 12 through 17 seem to contradict each other. First, the authors state that decreased brain AChE activity was observed in 21-day-old rat pups, but not in young adult female rats gavaged with tribufos at 5 mg/kg/day for 11 days. In the next statement, the authors report that the magnitude of decreased RBC AChE activity in the 21-day-old rats was similar to that observed in the young adult female rats. The authors need to revisit and clarify these points." (P9, L12-17 of annotated pages)

RESPONSE: *No revision necessary. The statements are not contradictory. One statement refers to RBC AChE activity and the other to brain AChE activity.*

Section 2.3. MINIMAL RISK LEVELS (MRLs)

COMMENT: The Reviewer stated "Estimates of minimal risk levels (MRLs) are often times based on the degree of RBC AChE inhibition induced by tribufos following exposure (acute or repeated) through different routes (oral or inhalation). It should be noted, however, that, as mentioned above, although RBC AChE inhibition is a validated biomarker of organophosphorus exposure, the degree of RBC AChE inhibition does not always correlate with the severity of the toxicity induced by organophosphorus compounds. This is particularly the case for the neurotoxic effects induced by continuous exposure to low levels of organophosphorus pesticides, which may be mediated by AChE-unrelated mechanisms and may differ among pesticides (please refer to last paragraph on page 2 of this review). Therefore, it is important to emphasize that in this toxicological profile, "less serious" effects are defined as: (i) effects not expected to cause significant dysfunction or death, (ii) effects whose toxicological significance to the organism is not entirely clear, or (iii) 20-59% AChE inhibition even in the absence of clinical signs of toxicity. "Serious" effects, on the other hand, are defined as: (i) effects associated with significant functional impairment, (ii) life-threatening effects, or (iii) ≥60% inhibition of neural or RBC AChE, even in the absence of clear clinical signs of intoxication (Chou and Williams-Johnson 1998). This was added to revised profile." (P11, L4-13; the Reviewer made similar suggestions for revision on P18, L7-19 of Chapter 3)

RESPONSE: *The paragraph describing seriousness of effects on RBC AChE activity was revised to state the following: In this Toxicological Profile, "less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose toxicological significance to the organism is not entirely clear. Serious effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). In addition to its presence and function in central and peripheral nervous tissue, AChE is also associated with red blood cells (RBCs). According to Chou and Williams-Johnson (1998), a 20–59% inhibition of neural or RBC AChE (i.e., 20–59%*

decrease in AChE activity) may be considered a less serious effect in the absence of more serious indicators of neurotoxicity. A ≥60% inhibition of neural or RBC AChE is considered a serious effect in the presence or absence of additional signs of neurotoxicity. However, the degree of RBC AChE inhibition does not always correlate with the severity of acute signs of organophosphorus toxicity, especially with respect to chronic exposure scenarios." The suggested discussion regarding correlation between organophosphorus pesticide-induced effects on AChE activity and severity of intoxication was added to Section 3.5.2 (Mechanisms of Toxicity).

COMMENT: P11, L21 and L23: The Reviewer stated that "Based on Table 4.1, Folex is a trade name of Tribufos. Yet, here folex appears to be something else. Please clarify."

RESPONSE: *The following clarification was added to the paragraph: "(formulations of folex previously used merphos, which is rapidly transformed in the environment to merphos oxide [tribufos])."*

COMMENT: P11, L30 and other locations in Chapter 2): The Reviewer stated "Please add age at which treatment began."

RESPONSE: *Chapter 2 is a short, high-level summary of effects reported in Chapter 3. The requested level of detail in Chapter 2 is beyond the scope of the chapter. See Chapter 3 for details.*

COMMENT: P12, L7: The Reviewer asked whether the clinical sign described as "decreased activity" refers to spontaneous locomotor activity.

RESPONSE: *No revision necessary. The statement regarding "decreased activity" was not further described in the available secondary source of information from the unpublished report.*

COMMENT: P13, L7: The Reviewer asked for a list of end points in a statement referring to NOAELs for developmental end points in selected animal studies.

RESPONSE: *No revision necessary. This level of detail is not considered necessary in Chapter 2. See Chapter 3 for details.*

COMMENT: P13, L7: The Reviewer asked for a list of end points in a statement referring to NOAELs for developmental end points in selected animal studies.

RESPONSE: *No revision necessary. This level of detail is not considered necessary in Chapter 2. See Chapter 3 for details.*

CHAPTER 3. HEALTH EFFECTS

Section 3.1 INTRODUCTION

COMMENT: The short introduction provides an adequate brief description of the primary objective of the profile.

RESPONSE: *No response is necessary.*

Section 3.2. DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

Toxicity -Quality of Humans Studies

COMMENT: "There are very few studies reporting the effects of tribufos in humans and the major limitations of the studies are described in the text without providing detailed discussions. Conclusions drawn from these studies seem to be appropriate and are accurately reflected in the profile. NOAELs and/or LOAELs could not be identified from the human study and the text provides adequate justification for excluding NOAELs/LOAELs. I am not aware of other studies with tribufos in humans."

RESPONSE: *No response is necessary.*

Toxicity -Quality of Animal Studies

COMMENT: "The effects of tribufos in animals have been assessed in a number of studies. Unfortunately, the toxicological endpoints are not always clearly presented in this profile and do not seem to justify some of the conclusions stated in the profile. Points that need clarification are identified in the revised version of the profile."

RESPONSE: *Responses to the Reviewer's "points that need clarification" are addressed in responses to specific comments below.*

COMMENT: "Where possible, NOAELs and LOAELs were identified for each study; however, not all toxicological effects are clearly described. For example, throughout the chapter the authors refer to neurotoxic effects without being specific about the endpoints being measured."

RESPONSE: *No revision necessary. Neurotoxic effects were typically clinical signs, which could also be interpreted as cageside observations as opposed to functional observational battery tests.*

COMMENT: "The profile does not make it clear as to how data were analyzed in the identified studies, making it difficult to assess the quality of the animal studies."

RESPONSE: *No revision necessary. Most animal study results were extracted from summaries (data evaluation records or reports) produced for EPA and released as cleared reviews; these summaries did not typically include detailed descriptions of methodology applied to data collection or analysis.*

Levels of Significant Exposure (LSE) Tables and Figures

COMMENT: "Tables and figures seem to be complete and self-explanatory and the "Users Guide" explains clearly how to use them. In the tables, it may helpful to parenthetically present M and F after doses. For instance, Table 3-1 shows that the NOAELs for endocrine effects derived from a study involving intermediate exposure to tribufos are 12.2 for males and 59.5 for females. It may be helpful to present these doses as 12.2 (M) and 59.5 (F) instead if 12.2 M and 59.5 F. This is particularly useful when the doses are integral numbers (e.g. first row in Table 3-1); in such cases, at first glance, it appears

that the table is referring to number of M and F in the study. As commented throughout the text, whenever available, the authors should consider adding to the tables the animals' age at which exposure began in any given study."

RESPONSE: *The original presentation of doses for males and females is considered appropriate and self-explanatory. Unless otherwise noted in the column "Species (strain) No./group," the test animals were adults. Additional age-related information does not appear necessary.*

Evaluation of Text

COMMENT: "Overall, major limitations of the studies are identified. A better description of the endpoints assessed in the studies and of the methods of analyses could substantially improve the reader's assessment of the conclusions drawn from the studies. In most instances, the endpoints have not been clearly defined or critically discussed in the profile."

RESPONSE: *No revision necessary. Limited study details were available in most of the publicly-available sources of information from unpublished studies (mostly data evaluation records [DERs] from EPA). Sources were reviewed to ensure that pertinent details were included in the toxicological profile.*

COMMENT: "'Bottom-line' statements and 'take-home' messages appear throughout the profile. However, they are not always substantiated by the data presented in the profile – this is particularly true for conclusions that no additional animal study is needed to assess the potential of tribufos to induce neurotoxic effects."

RESPONSE: *The profile was reviewed for concluding statements. In particular, the statement regarding the lack of need for additional animal data was replaced with the following statement: "Additional developmental toxicity studies in animals should be designed to evaluate possible tribufos-related effects on behavior and cognitive function."*

COMMENT: "Wherever possible, dose-response relationships have been addressed. Animal data are the primary basis used to draw support for human effects, because human studies are largely inexistent. However, the authors clearly discuss the limitations of extrapolations and identify the need for assessment of human populations exposed to tribufos."

RESPONSE: *No response is necessary.*

COMMENT: "This chapter has been revised. Clarification is often requested on the following topics: age of animals at time of testing, sex of animals, and types of tests animals are subjected to."

RESPONSE: *No revision necessary. The requested information is not considered necessary and is not available in many of the publicly-available sources that serve as summaries of unpublished studies.*

Section 3.3 GENOTOXICITY

COMMENT: "This section appears to be adequately developed given the limited data available in the literature. Please describe whether the S9 mix used in the studies here are derived from human or rat liver."

RESPONSE: *See response to the specific comment on annotated pages (identified as P38, L5 Section 3.3 Genotoxicity).*

Section 3.4 TOXICOKINETICS

COMMENT: "The issues of absorption, distribution, metabolism, and excretion of tribufos are well discussed. Major organs and tissues to which tribufos distributes have been identified. All known metabolites and metabolic parameters have been presented. Unfortunately, no pharmacokinetic/ pharmacodynamic models are available for tribufos. In addition, differences in toxicokinetics between humans and animals could not be discussed due to lack of PK data in humans. The need for additional studies is adequately identified and justified. Minor comments are as follows:
Age and sex of animals are missing throughout the description of the studies on the toxicokinetics of tribufos."

RESPONSE: *No revision necessary. This Toxicological Profile for Tribufos is intended as a high-level summary of toxicological, chemical, and environmental issues. The level of detail requested in the toxicokinetics section is beyond the scope of this high-level summary document.*

Section 3.5 MECHANISMS OF ACTION

COMMENT: "It is well accepted that AChE inhibition is the primary mechanism of action underlying the acute toxicity of organophosphorus compounds, including tribufos. The authors discussed this mechanism in the profile. However, it is well acknowledged in the scientific literature that mechanisms other than AChE inhibition contribute to the toxic effects of organophosphorus compounds (reviewed in Terry, 2012; Pereira et al., 2014). This section has been revised accordingly in the profile. Pertinent references are also attached as pdf."

RESPONSE: *This section was revised to read: "AChE-unrelated mechanisms, which are likely to differ from one organophosphorus compound to another, have been proposed to explain the effects of long-term exposure to low levels. Organophosphorus compounds can directly interact with nicotinic and muscarinic receptors (Albuquerque et al. 1985; Bomser and Casida 2001; Jett et al. 1991) and structural proteins such as tubulin, kinesin, and dynein (Androutsopoulos et al. 2013; Terry 2012). These and other non-AChE mechanisms, including exacerbated oxidative stress (Garry 2004; Ray 1998), imbalanced intracellular Ca2+ homeostasis, increased signaling mediated by inflammatory mediators such as interleukins and cytokines, changes in cellular signaling mediated by neurotrophin receptors and protein kinases, and mitochondrial disruption, have been proposed to contribute to the toxicity of organophosphorus compounds (Androutsopoulos et al. 2013; Banks and Lein 2012; Terry 2012). However, no information was located to suggest that such non-AChE mechanisms are involved in tribufos toxicity."*

Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

COMMENT: "It is unclear whether the effects of tribufos in the adrenal glands observed in CD-1 mice exposed through their diet to the pesticide, already described on page 24 under "Endocrine Effects," belong in this section. These are not toxicities mediated through the endocrine axis. Instead, they are toxic endocrine effects."

RESPONSE: *The text in question was deleted from Section 3.6.*

Section 3.7 CHILDREN'S SUSCEPTIBILITY

COMMENT: "This chapter begins with an extensive discussion of how children may be more sensitive to different toxicants. Surprisingly, however, it does not review the data that are available in the literature regarding the developmental neurotoxic effects of organophosphorus pesticides. Epidemiological studies have provided evidence that organophosphorus pesticides are toxic to the developing human brain (Bouchard et al., 2011; Rauh et al., 2012; Rosas and Eskenazi, 2008). Specifically, increased prevalence of psychomotor and cognitive deficits among children ages 2 to 7 have been associated with prenatal exposure to levels of organophosphorus pesticides that do not trigger overt signs of acute intoxication (Bouchard et al., 2011; Horton et al., 2012; Perera et al., 2003; Rauh et al., 2006; Whyatt et al., 2004). The effects of prenatal exposure to pesticides on cognition in children are sexually dimorphic, with boys being more affected than girls (Rauh et al., 2012). In addition, imaging studies have provided evidence that the detrimental effects of prenatal exposure to organophosphorus pesticides on cognition in children correlate with disruption of the structural integrity of a number of brain regions (Rauh et al., 2012). Preclinical studies in a number of animal models have supported the notion that the developing brain is exquisitely sensitive to the neurotoxic effects of organophosphorus pesticides. There is general consensus in all preclinical studies in the literature that cognitive impairment associated with developmental exposure to the organophosphorus pesticide chlorpyrifos is sexually dimorphic. In general, cognitive deficits resulting from neonatal exposure of rats or mice and prenatal exposure of guinea pigs to the organophosphorus pesticide chlorpyrifos are more pronounced among males than females (Aldridge et al., 2005; Johnson et al., 2009; Levin et al., 2001; Mamczarz et al., 2016). The postnatal period of exposure of rats and mice to the pesticide corresponds to the period of brain growth spurt, a phenomenon that is largely postnatal in rats and mice and prenatal in guinea pigs and humans (Dobbing and Sands, 1970).

This information is added to the profile for the authors' consideration. Additional revisions are included in this section of the profile as well."

RESPONSE: *ATSDR relayed information on tribufos animal studies and the sensitivity of neonates. This included psychomotor effects. Most of the Reviewer's comments appear to be about chloropyrifos and that is not the chemical being profiled.*

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

COMMENT: "This section provides a general idea of pharmacodynamic and pharmacokinetic means by which different substances could affect the activity of tribufos. In the absence of studies using tribufos to assess drug-drug interactions, this section seems to be appropriately developed."

RESPONSE: *No response is necessary.*

Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

COMMENT: "This section can be expanded to refer to the high susceptibility of the developing brain to organophosphorus pesticides already discussed in section 3.7. and to introduce the usefulness of paraoxonase (PON1) as a potential biomarker of susceptibility for organophophorus intoxication. Mice with a null mutation in the gene that encodes PON1 are significantly more sensitive to organophosphorus intoxication than their wild type counterpart. In addition, low levels of serum PON1 activity in infants and children have been proposed to underlie the high sensitivity of this sector of the population to the neurotoxic effects of organophosphorus pesticides. A discussion of the subject can be found in Costa et al (2003). The profile has been edited accordingly."

RESPONSE: *See response to the specific comment on annotated pages (identified as P38, L19-29; Section 3.10 Populations that are Unusually Susceptible).*

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

COMMENT: "Management and treatment of acute intoxication is not specific for tribufos. Instead, it applies to any cholinergic crisis induced by drugs/toxicants that inhibit AChE. Conventional antidotal therapy to treat organophosphorus intoxication includes the use of atropine to block overactivation of muscarinic receptors, and oximes (mostly pralidoxime, aka 2-PAM) to reactivate organophosphorus-inhibited AChE that has not aged. The authors may consider including in this profile information that if patients develop convulsions, anticonvulsants (generally midazolam or diazepam) may be prescribed. Limitations of the conventional antidotal therapy have been reported. The authors may consider adding information that 2-PAM is not an effective antidote against all organophosphorus compounds. In addition, 2-PAM does not cross the blood brain barrier, and, as such, it is ineffective at reversing the neurotoxic effects of organophophorus compounds. After evaluating clinical outcomes, Buckley et al. (2011) have concluded that current clinical evidence is not sufficient to indicate whether oximes are harmful or beneficial as antidotes against organophosphorus intoxication. This information has been added to the revised profile."

RESPONSE: *See response to the specific comment on annotated pages (identified as P60, 13-14; Section 3.11.3 Interfering with the Mechanism of Action for Toxic Effects through P60, L34 to P61, L4; Section 3.11.3 Interfering with the Mechanism of Action for Toxic Effects).*

Section 3.12 ADEQUACY OF THE DATABASE

COMMENT: "Throughout this section, the authors state repeatedly that several studies included evaluations of neurological end points. The authors conclude that the potential for tribufos to cause systemic or neurological effects has been adequately assessed for inhalation, oral, and dermal exposure routes. Unfortunately, their conclusion is not substantiated by the data presented in the profile. Neurological endpoints for toxicological studies are well defined in the literature. As described in Dr. Virginia Moser's review (2011), functional tests used for evaluation of neurotoxicity assess different behavioral repertoires that range from a first-tier, hazard identification based on screening analysis of spontaneous activity and cageside observations to a second-tier, hazard characterization based on assessment of cognitive, motor, and sensory functions. According to the "Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies" published by the EPA in 2013, a "neurotoxicity battery [(i.e., acute (ACN) and subchronic (SCN) studies)] are required for all conventional food and non-food pesticides." According to the EPA guidance, the studies need to assess the effects of the pesticides on scientific endpoints related to the functions of the

peripheral and central nervous systems, and, as such, the studies should include functional tests that assess neuromuscular, sensory, and cognitive functions, in addition to histopathology of the nervous systems. The data presented in this profile provide no quantitative information on the potential effects of tribufos on cognitive, sensory, or motor functions. Most assessments of neurological function seem to be restricted to cageside observations. Therefore, it seems premature to conclude that potential for tribufos to induce neurological effects has been adequately assessed."

RESPONSE: *See response to the specific comment on annotated pages (identified as P35, L17-18; Section 3.2.2.6 Developmental Effects).*

Section 3.12.2. Identification of Data Needs

COMMENT: "The authors need to revisit their conclusion that 'The potential for tribufos to cause systemic or neurological effects has been adequately assessed for inhalation, oral, and dermal exposure routes.' Based on the data presented in this profile, on the guidelines published by the EPA, and on scientific knowledge in the field, one would conclude that animal studies are necessary to assess the potential for exposure to tribufos at different ages (including prenatal) to affect neurological functions. In addition, there is no scientific basis to justify the assertion that 'it is not likely that tribufos-specific biomarkers of effect exist.'"

RESPONSE: *See response to the specific comment on annotated pages (identified as P64, L12-14*; *Section 3.12.2 Intermediate-Duration Exposure through P66, L22-23; Section 3.12.2 Biomarkers of Exposure and Effect).*

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

COMMENT: "This chapter is well developed. I am not aware of information or values that are wrong or missing in this chapter and its tables."

RESPONSE: *No response is necessary.*

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

COMMENT: "This chapter seems to be adequately developed. I am not aware of any information that is wrong or missing in this chapter."

RESPONSE: *No response is necessary.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

COMMENT: "This chapter is well developed. The text appropriately traces the substance from its point of release to the environment until it reaches the target population. Sufficient and technically sound information regarding the extent of occurrence of tribufos at NPL sites is provided. The text covers pertinent information relative to transport, partitioning, transformation, and degradation of tribufos. The text also provides adequate information on levels monitored or estimated in the environment, with proper units being used for each medium. There is an adequate description of sources of exposure for the general population and occupations involved in handling of tribufos, as well as populations with potentially high

exposures and/or sensitivity. Finally, most data needs are adequately justified. It appears, however, that tribufos levels have not been measured in milk of lactating mothers and in maternal/fetal cord blood obtained from individuals living near or working in sites where tribufos is sprayed. This is an important piece of information needed for adequate assessment of the potential for exposure of developing fetuses/infants to tribufos."

RESPONSE: *ATSDR has added this as a data need in Section 6.8.1.*

CHAPTER 7. ANALYTICAL METHODS

COMMENT: "Overall, this chapter seems to be well developed. I am unaware of additional methods that can be added to the tables. There are no unique issues related to sampling tribufos. The data needs are presented in an unbiased, neutral, and non-judgmental fashion."

RESPONSE: *No response is necessary.*

CHAPTER 8. REGULATIONS AND ADVISORIES

COMMENT: "Overall, this chapter seems to be well developed. I am unaware of other regulations or guidelines that may be appropriate for the table."

RESPONSE: *No response is necessary.*

CHAPTER 9. REFERENCES

COMMENT: "A pdf of each reference mentioned in this review is attached to the message. These references are also listed here.

Aldridge JE, Levin ED, Seidler FJ, Slotkin TA. 2005. Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. Environ Health Perspect 113:527-531.

Ames RG, Brown SK, Mengle DC, Kahn E, Stratton JW, Jackson RJ. 1989. Cholinesterase activity depression among California agricultural pesticide applicators. Am J Ind Med 15:143-150.
Androutsopoulos VP1, Hernandez AF, Liesivuori J, Tsatsakis AM. 2013. A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides. Toxicology 307:89-94.

Banks CN1, Lein PJ. 2012. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology 33:575-584.

Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, Trujillo C, Johnson C, Bradman A, Barr DB, Eskenazi B. 2011. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect 119:1189-1195.

Buckley NA, Eddleston M, Li Y, Bevan M, Robertson J. 2011. Oximes for acute organophosphate pesticide poisoning. Cochrane Database Syst Rev (2):CD005085.

Costa LG, Giordano G, Cole TB, Marsillach J, Furlong CE. 2013. Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. Toxicology 307:115-122.

D'Alessandro A, Liumbruno G, Grazzini G, Zolla L. 2010. Red blood cell storage: the story so far. Blood Transfus 8:82-88.

Dobbing J, Sands J. 1979. Comparative aspects of the brain growth spurt. Early Hum Dev 3:79-83.

EPA 2013. Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies.

Farahat FM, Ellison CA, Bonner MR, McGarrigle BP, Crane AL, Fenske RA, Lasarev MR, Rohlman DS, Anger WK, Lein PJ, Olson JR. 2011. Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers. Environ Health Perspect 119:801-806.

Gunier RB, Bradman A, Harley KG, Kogut K, Eskenazi B. 2016. Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-Year-Old Children. Environ Health Perspect. PMID 27453326.

Horton MK, Kahn LG, Perera F, Barr DB, Rauh V. 2012. Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? Neurotoxicol Teratol 34:534-541.

Johnson FO, Chambers JE, Nail CA, Givaruangsawat S, Carr RL. 2009. Developmental chlorpyrifos and methyl parathion exposure alters radial-arm maze performance in juvenile and adult rats. Toxicol Sci 109:132-142.

Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA. 2001. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Dev Brain Res 130:83-89.

Levy-Khademi F, Tenenbaum AN, Wexler ID, Amitai Y. 2007. Unintentional organophosphate intoxication in children. Pediatr Emerg Care 23:716-718.

Mamczarz J, Pescrille JD, Gavrushenko L, Burke RD, Fawcett WP, DeTolla LJ Jr, Chen H, Pereira EFR, Albuquerque EX. 2016. Spatial learning impairment in prepubertal guinea pigs prenatally exposed to the organophosphorus pesticide chlorpyrifos: Toxicological implications. Neurotoxicology 56:17-28.

Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. 2010. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ Health Perspect 118:1768-1774.

Mason HJ. 2000. The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos. Occup Med (Lond) 50:343-347.

Moser VC. 2011. Functional assays for neurotoxicity testing. Toxicol Pathol 39:36-45.

Pereira EFR, Aracava Y, DeTolla LJ Jr, Beecham EJ, Basinger GW Jr, Wakayama EJ, Albuquerque EX. 2014. Animal models that best reproduce the clinical manifestations of human intoxication with organophosphorus compounds. J Pharmacol Exp Ther 350:313-321.

Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, Bernert T, Garfinkel R, Tu YH, Diaz D, Dietrich J, Whyatt RM. 2003. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect. 111:201-205.

Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118:e1845-1859.

Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Slotkin TA, Peterson BS. 2012. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc Natl Acad Sci USA 109:7871-7876.

Rauh VA, Garcia WE, Whyatt RM, Horton MK, Barr DB, Louis ED. 2015. Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. Neurotoxicology 51:80-86.

Rosas LG, Eskenazi B. Pesticides and child neurodevelopment. 2008. Curr Opin Pediatr 20:191-197.

Ross SM, McManus IC, Harrison V, Mason O. 2013. Neurobehavioral problems following low-level exposure to organophosphate pesticides: a systematic and meta-analytic review. Crit Rev Toxicol 43:21-44.

Silman I, Sussman JL. 2005. Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Curr Opin Pharmacol 5:293-302.

Singleton ST, Lein PJ, Dadson OA, McGarrigle BP, Farahat FM, Farahat T, Bonner MR, Fenske RA, Galvin K, Lasarev MR, Anger WK, Rohlman DS, Olson JR. 2015. Longitudinal assessment of occupational exposures to the organophosphorous insecticides chlorpyrifos and profenofos in Egyptian cotton field workers. Int J Hyg Environ Health 218:203-211.

Stein LJ, Gunier RB, Harley K, Kogut K, Bradman A, Eskenazi B. 2016. Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort. Neurotoxicology 56:180-187.

Strelitz J, Engel LS, Keifer MC. 2014. Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers. Occup Environ Med 71:842-847.

Terry AV Jr. 2012. Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms. Pharmacol Ther 134:355-365.

Wenthold RJ, Mahler HR, Moore WJ. 1974. The half-life of acetylcholinesterase in mature rat brain. J Neurochem 22:941-943.

Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, Hoepner LA, Diaz D, Dietrich J, Reyes A, Tang D, Kinney PL, Perera FP. 2004. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect 112:1125-1132."

RESPONSE: *All identified studies were reviewed for pertinent information. All information deemed relevant to the toxicological profile for tribufos was added.*

Specific Comments on Annotated Pages

Specific comments on annotated pages of Chapters 1 and 2 are addressed in the corresponding sections above. Specific comments on annotated pages of Chapters 3-9 are addressed below.

Section 3.2. DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

COMMENT: P16, L29-31: The Reviewer suggested adding the following to boilerplate text: "**In the absence of clear clinical signs of intoxication, 20-59% inhibition of RBC AChE activity is used to define "less serious" effects and >60% inhibition of RBC AChE activity is used to define "serious" effects.**"

RESPONSE: *The suggested addition was not made because similar definitions are provided in the following text. The boilerplate text is designed to be applicable to each toxicological profile independent of the specific substance or substances included in a particular toxicological profile.*

COMMENT: P17, L23 to P18, L10: The Reviewer made several suggestions for changes to more specifically describe the principal effect of tribufos (AChE inhibition) and ATSDR's consideration of the inhibition as an indicator of an adverse neurological effect.

RESPONSE: *The suggested changes were made.*

COMMENT: P19, L2 (Section 3.2.1.1 Death): "Please include the animal at which rats were exposed to tribufos."

RESPONSE: *The animal is already listed on the page as "Sprague-Dawley rats."*

COMMENT: P19, L8 (Section 3.2.1.1 Death): "If available, please add the age at which the animals were exposed to tribufos."

RESPONSE: *The age of the rats (2–3 months) was added to the text.*

COMMENT: P19, L31 (Section 3.2.1.2 Respiratory Effects): "Please spell out RR. It is not defined in the list of acronyms either."

RESPONSE: *RR was spelled out and added to the list of acronyms.*

COMMENT: P20, L13 (Section 3.2.1.2 Respiratory Effects): Regarding the statement: "The proportions of respiratory-caused mortality (number of deaths due to respiratory causes during the cotton defoliation period of each year divided by the respiratory deaths during the rest of that year in cotton growing areas divided by a similar proportion of respiratory cause mortality in non-cotton growing areas) ranged from 0.798 to 1.153 and exhibited a statistically significant ($p<0.05$) pattern of increases for 15 of the 21 years", the Reviewer stated: "In the absence of an explanation of how the analysis was perfumed, the p value is meaningless."

RESPONSE: *No revision necessary. The p-value is considered informative; it does not appear necessary to include specific details regarding analysis methodology in this high-level summary toxicological profile.*

COMMENT: P26, L27 and 30 (Section 3.2.1.2 Other Systemic Effects): The Reviewer asked whether hypothermia was defined as "cold to the touch" in the study.

RESPONSE: *No revision necessary. Yes.*

COMMENT: P26, L29 (Section 3.2.1.2 Other Systemic Effects): The Reviewer asked whether hypothermia was observed immediately after the administration of tribufos.

RESPONSE: *The sentence in question was revised to note that "cold to the touch" was observed as early as 4 hours postdosing.*

COMMENT: P27, L21 (Section 3.2.2.4 Neurological Effects): The Reviewer stated: "RBC AChE activity is not a true measurement of neurotoxicity."

RESPONSE: *Agreed; for that reason, it was referred to as an "indicator" of neurological effects.*

COMMENT: P32, L14-15 (Section 3.2.2.4 Neurological Effects): Regarding a statement "Mice are somewhat less sensitive than rats and dogs to tribufos-related effects on AChE activity following intermediate-duration oral exposure", the Reviewer stated "Are the animals exposed to tribufos at ages that are equivalent? In addition, it seems that dogs were exposed to tribufos for much longer than mice or rats. Even considering the different life span of the species, the comparison does not seem to be appropriate. This may need to be elaborated."

RESPONSE: *The statement in question was revised to read "Mice appear to be somewhat less sensitive…".*

COMMENT: P33, 6+ (Section 3.2.2.4 Neurological Effects): Regarding statements that oral dosing with tribufos resulted in "decreased activity", the Reviewer asked for verification as to whether "decreased activity" refers to "decreased spontaneous locomotor activity".

RESPONSE: *The statement regarding "decreased activity" was revised to "appearance of decreased activity" as was stated in the available EPA DER summaries of the unpublished studies.*

COMMENT: P33, L14-15 (Section 3.2.2.4 Neurological Effects): Regarding a statement "No clinical signs…", the Reviewer stated "Please be more specific. Is this statement based on cageside observations only? Assessment of clinical signs of neurotoxicity include subjecting the animals to a battery of neurobehavioral (cognitive and non-cognitive) tests."

RESPONSE: *The text was revised to note that the clinical signs were based on cageside observations.*

COMMENT: P34, L27-29 (Section 3.2.2.6 Developmental Effects): Regarding a statement "In another study of Sprague-Dawley rat dams gavaged during GDs 6–19, the only apparent developmental effect was significantly lower mean male fetal body weight (6% lower than that of controls) at 28 mg/kg/day (EPA 2012f)", the Reviewer stated "This statement is somewhat misleading. It conveys the notion that the animals were subjected to a comprehensive battery of tests. Was that the case, or are the animals only subjected to cageside observations and body weight measurements?"

RESPONSE: *The text was revised to state "In another study of Sprague-Dawley rat dams gavaged during GDs 6–19, there were no signs of treatment-related fetal effects, with the exception of significantly lower mean male fetal body weight (6% lower than that of controls) at 28 mg/kg/day (EPA 2012f)."*

COMMENT: P35, L13-14 (Section 3.2.2.6 Developmental Effects): Regarding a statement "…decreased motor activity at postnatal day (PND) 13 (males) and …", the Reviewer asked "Were all the other effects described in this paragraph observed in males and females?"

RESPONSE: *The statement "… decreased locomotor activity at postnatal day (PND) 13 (males) and…" was deleted because it was in error. The remaining effects were observed in males and females, with the exception of delayed preputial separation (a landmark applicable to males only).*

COMMENT: P35, L17-18 (Section 3.2.2.6 Developmental Effects): The Reviewer stated "It is worth to mention that no study has assessed the effects of developmental exposure to tribufos on cognitive functions and on anxiety- and depression-related behaviors."

RESPONSE: *The statement "The neurotoxicity of tribufos in animals has been adequately assessed." was deleted from the data needs section of the profile. The following statement was added to the data needs section of the profile: "Additional developmental toxicity studies in animals could be designed to evaluate possible tribufos-related effects on behavior and cognitive function."*

COMMENT: P36, L35 (Section 3.2.3.1 Death): The Reviewer asked whether "decreased activity" refers to spontaneous locomotor activity.

RESPONSE: *No revision necessary. The term "decreased activity" was not more clearly defined in the available DER of the study.*

COMMENT: P37, L13-14 (Section 3.2.3.2 Hematological Effects): Regarding the statement "Hematological evaluations made just prior to the initiation of treatment and following the final treatment revealed no signs of tribufos-induced hematological effects at any dose level.", the Reviewer stated "Please list which hematological evaluations were performed in the study."

RESPONSE: *The statement was revised to read "There were no signs of tribufos-induced effects on RBCs, WBCs, platelets, hemoglobin, or hematocrit at any dose level."*

COMMENT: P38, L4 (Section 3.2.3.4 Neurological Effects): The Reviewer stated "Based on Table 4.1, Folex is a trade name of Tribufos. Yet, here folex appears to be something else. Please clarify."

RESPONSE: *In response to a previous comment, the following text was added to describe folex: "(formulations of folex previously used merphos, which is rapidly transformed in the environment to merphos oxide [tribufos])."*

COMMENT: P38, L8 (Section 3.2.3.4 Neurological Effects): Regarding the statement "There were no clinical signs of exposure-related neurotoxicity…", the Reviewer stated "What does this mean? Is this statement based on cageside observations only or on a battery of behavioral tests?"

RESPONSE: *The statement was revised to read "There were no clinical signs of exposure-related neurotoxicity or effects on peripheral nerve function or neuromuscular transmission,…".*

COMMENT: P38, L5 (Section 3.3 Genotoxicity): The Reviewer stated "In the studies described here is the S9 mix derived from rat or human liver?"

RESPONSE: *The text was revised to note that rat liver S9 mix was employed in the study of Chen et al.; the source of exogenous metabolic activation was not specified in the available secondary source (CalEPA 2004) of the other studies summarized in Section 3.3 of the draft toxicological profile for tribufos.*

COMMENT: P48, L25-29 (Section 3.5.2 Mechanisms of Toxicity): The Reviewer suggested adding the following text: "Numerous studies have also provided evidence of non-enzymatic functions mediated by AChE that include but are not restricted to axonal outgrowth (Bigbee et al., 2000), synaptogenesis (Sternfeld et al., 1998), cell adhesion (Bigbee et al., 2004), and neuronal migration (Dori et al., 2005). These non-enzymatic actions of AChE appear to be especially critical for synaptic development (reviewed in Silman and Sussman, 2005)."

RESPONSE: *The suggested information was added to Section 3.5.2 (Mechanisms of Toxicity).*

COMMENT: P48, L31 to P49, L28 (Section 3.5.2 Mechanisms of Toxicity): The Reviewer suggested deleting the following text: "As an anticholinesterase organophosphate, tribufos inhibits AChE by reacting with the active site to form a stable phosphorylated complex incapable of destroying acetylcholine at the synaptic gutter between the pre- and postsynaptic nerve endings or neuromuscular junctions of skeletal muscles resulting in accumulation of acetylcholine at these sites. This leads to continuous or excessive stimulation of cholinergic fibers in the postganglionic parasympathetic nerve endings, neuromuscular junctions of the skeletal muscles, and cells of the central nervous system that results in hyperpolarization and receptor desensitization. These cholinergic actions involving end organs (heart, blood vessels, secretory glands) innervated by fibers in the postganglionic parasympathetic nerves result in muscarinic effects, which are manifested as miosis, excessive glandular secretions (salivation, lacrimation, rhinitis), nausea, urinary incontinence, vomiting, abdominal pain, diarrhea, bronchoconstriction or bronchospasm, increased bronchosecretion, vasodilation, bradycardia, and hypotension. Nicotinic effects are due to accumulation of acetylcholine at the skeletal muscle junctions and sympathetic preganglionic nerve endings. Nicotinic effects are manifested as muscular fasciculations, weakness, mydriasis, tachycardia, and hypertension. The central nervous system effects are due to accumulation of acetylcholine at various cortical, subcortical, and spinal levels (primarily in the cerebral cortex, hippocampus, and extrapyramidal motor system). The central nervous system effects are manifested as respiratory depression, anxiety, insomnia, headache, restlessness, tension, mental confusion, loss of concentration, apathy, drowsiness, ataxia, tremor, convulsion, and coma. Oxidative stress has been proposed as an additional mechanism of

action for organophosphorus pesticides such as tribufos (Garry 2004; Ray 1998)." The Reviewer suggested replacing the deleted text with the following: "Tribufos inhibits AChE by reacting with residues in the active site of the enzyme to form a stable phosphorylated complex incapable of metabolizing acetylcholine. This leads to continuous or excessive stimulation of cholinergic receptors (nicotinic and muscarinic) in the peripheral and central nervous systems. Overactivation of muscarinic receptors in eyes, heart, blood vessels, respiratory tract, gastrointestinal tract, and secretory glands results in miosis, excessive glandular secretions (salivation, lacrimation, rhinitis), nausea, urinary incontinence, vomiting, abdominal pain, diarrhea, bronchoconstriction or bronchospasm, increased bronchosecretion, vasodilation, bradycardia, and hypotension. Overstimulation of nicotinic receptors at the skeletal muscle junctions and sympathetic preganglionic nerve endings leads to muscle fasciculations, weakness, mydriasis, tachycardia, and hypertension. The central nervous system effects are due to accumulation of acetylcholine at various cortical, subcortical, and spinal levels (primarily in the cerebral cortex, hippocampus, and extrapyramidal motor system). The central nervous system effects include respiratory depression, anxiety, insomnia, headache, restlessness, tension, mental confusion, loss of concentration, apathy, drowsiness, ataxia, tremor, convulsion, and coma."

RESPONSE: *No revision necessary. The suggested replacement of text was not made; the original text is considered adequate with citations.*

COMMENT: P49, L30 to P50, L4 (Section 3.5.2 Mechanisms of Toxicity): The Reviewer suggested adding the following text: "In addition to blocking AChE, organophosphorus compounds such as tribufos can directly interact with nicotinic and muscarinic receptors (Albuquerque et al., 1985; Bomser and Casida, 2001; Jett et al., 1992) and structural proteins such as tubulin, kinesin, and dynein (Androustsopoulos et al., 2013; Terry, 2012). These and other non-AChE mechanisms, including exacerbated oxidative stress (Garry 2004; Ray 1998), imbalanced intracellular Ca2+ homeostasis, increased signaling mediated by inflammatory mediators such as interleukins and cytokines, changes in cellular signaling mediated by neurotrophin receptors and protein kinases, and mitochondrial disruption, have been proposed to contribute to the toxicity of organophosphorus compounds (reviewed in Androustsopoulos et al., 2013; Banks and Lein, 2012; Terry, 2012)."

RESPONSE: *The suggested text was added to Section 3.5.2 (Mechanisms of Toxicity).*

COMMENT: P51, L12-23 (Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS): The Reviewer stated "the effects of tribufos in the adrenal glands observed in CD-1 mice exposed through their diet to the pesticide, already described on page 24 under "Endocrine Effects," belong in this section. These are not toxicities mediated through the endocrine axis. Instead, they are toxic endocrine effects."

RESPONSE: *The information the Reviewer highlighted regarding tribufos-related effects on adrenal glands of laboratory animals was deleted because the effect was not part of the endocrine axis and endocrine function was not assessed.*

COMMENT: P53, L32 to P54, L18 (Section 3.7 Children's Susceptibility): The Reviewer suggested adding the following text: "However, epidemiological studies have provided evidence that organophosphorus pesticides such as tribufos are toxic to the developing human brain (Bouchard et al., 2011; Rauh et al., 2012; Rosas and Eskenazi, 2008). Specifically, increased prevalence of psychomotor and cognitive deficits among children ages 2 to 7 and childhood tremors have been associated with prenatal exposure to levels of organophosphorus pesticides that do not trigger overt signs of acute

intoxication (Bouchard et al., 2011; Gunier et al., 2016; Marks et al., 2010; Rauh et al., 2012, 2015; Rosas and Eskenazi, 2008; Stein et al., 2016). The effects of prenatal exposure to pesticides on cognition in children are sexually dimorphic, with boys being more affected than girls (Horton et al., 2012; Rauh et al., 2012). In addition, imaging studies have provided evidence that the detrimental effects of prenatal exposure to organophosphorus pesticides on cognition in children correlate with disruption of the structural integrity of a number of brain regions (Rauh et al., 2012).

Preclinical studies in a number of animal models have supported the notion that the developing brain is exquisitely sensitive to the neurotoxic effects of organophosphorus pesticides. There is general consensus in all preclinical studies in the literature that cognitive impairment associated with developmental exposure to the organophosphorus pesticide chlorpyrifos is sexually dimorphic. In general, cognitive deficits resulting from neonatal exposure of rats or mice and prenatal exposure of guinea pigs to the organophosphorus pesticide chlorpyrifos are more pronounced among males than females (Aldridge et al., 2005; Johnson et al., 2009; Levin et al., 2001; Mamczarz et al., 2016). The postnatal period of exposure of rats and mice to the pesticide corresponds to the period of brain growth spurt, a phenomenon that is largely postnatal in rats and mice and prenatal in guinea pigs and humans (Dobbing and Sands, 1979)."

RESPONSE: *The information regarding psychomotor and cognitive deficits and tremors in children was added to Section 3.7 (Children's Susceptibility). Information regarding male/female differences was added to Section 3.10 (Populations that are Unusually Susceptible).*

COMMENT: P54, L28 (Section 3.7 Children's Susceptibility): The Review asked for clarification of the statement "clinical signs of neurotoxicity" (i.e., whether it was based on cageside observations or a battery of neurobehavioral tests).

RESPONSE: *The word "clinical" was replaced with "cageside."*

COMMENT: P54, L30-31 (Section 3.7 CHILDREN'S SUSCEPTIBILITY): In response to the statement "No additional information was located regarding possible age-related differences in tribufos toxicity in laboratory animals", the Reviewer stated "In Chapter 2, the authors reported that decreased brain AChE activity was observed in 21-day-old rat pups, but not in young adult female rats gavaged with tribufos at 5 mg/kg/day for 11 days. These would be age-dependent differences in the effects of tribufos. Please elaborate."

RESPONSE: *The information regarding age-related differences in tribufos-induced effects on brain AChE activity was added to the section. The statement regarding no additional information was deleted.*

COMMENT: P56, L6-9 (Section 3.8.1 Biomarkers Used to Identify or Quantify Exposure to Tribufos): The Reviewer stated "The authors could introduce the fact that dialkyl phosphate metabolites in different matrices (including urine, feces, and meconium) have been used as biomarkers of organophosphorus pesticide exposure (reviewed in Wessels et al., 2003). The authors could report that measurements of RBC AChE activity are commonly used to assess potential exposure to organophosphorus pesticides such as tribufos. They could also introduce that given its high affinity to orgaphosphorus compounds, plasma butyrylcholinesterase (BuChE) is also used as a biomarker of exposure to these chemicals. It is important to note, however, neither AChE nor BuChE is specific for any given organophosphorus compound."

RESPONSE: *Information regarding activity of the enzymes BuAChE and AChE in the blood as a biomarker of effect (section 3.8.2) was added with the caveat that these markers are not specific to tribufos.*

COMMENT: P56, L13-22 (Section 3.8.2 Biomarkers Used to Characterize Effects Caused by Tribufos): The Reviewer stated "The authors could remove these statements altogether, as they have already been introduced at least twice in previous sections. Instead, they could discuss the limitations of the use of measurements of RBC AChE activity as a biomarker of effect."

RESPONSE: *No revision necessary. These statements were not removed because they are pertinent to the section.*

COMMENT: P58, L19-29 (Section 3.10 Populations that are Unusually Susceptible): The Reviewer suggested adding the following: "Recent studies have also proposed that paraoxonase (PON1) may be a potential biomarker of susceptibility for organophophorus intoxication. Mice with a null mutation in the gene that encodes PON1 are significantly more sensitive to organophosphorus intoxication than their wild type counterpart. In addition, low levels of serum PON1 activity in infants and children have been proposed to underlie the high sensitivity of this sector of the population to the neurotoxic effects of organophosphorus pesticides. An in-depth discussion of the subject can be found in Costa et al (2003).

As discussed in section 3.7., the developing brain is known to be more sensitive to the untoward effects of a number of toxicants, including organophosphorus pesticides such as tribufos. Unfortunately, to date, no clinical or preclinical study has assessed the impact of prenatal exposure to tribufos on cognitive functions, anxiety- and depression-related behaviors in childhood or adulthood."

RESPONSE: *The suggested addition of data regarding PON1 as a potential biomarker of susceptibility to organophosphorus intoxication is considered too speculative. The suggested changes were not made.*

COMMENT: P60, 13-14 (Section 3.11.3 Interfering with the Mechanism of Action for Toxic Effects): The Reviewer suggested adding the following: "If patients develop convulsions, anticonvulsants (generally midazolam or diazepam) may be used."

RESPONSE: *The Reviewer statement was added, verbatim, to the section 3.11.*

COMMENT: P60, L14-17 (Section 3.11.3 Interfering with the Mechanism of Action for Toxic Effects): Regarding the statement "It should be mentioned, however, that glycopyrrolate, a quaternary ammonium compound, has also been used instead of atropine (Bardin and Van Eeden 1990). Unlike atropine, glycopyrrolate does not cross the blood-brain barrier and, therefore, has fewer central nervous system effects.", the Reviewer stated "The use of a glycopyrrolate is not recommended for treatment of acute organophosphorus intoxication."

RESPONSE: *The suggested addition was not made because glycopyrrolate treatment is of questionable usefulness.*

COMMENT: P60, 17-18 (Section 3.11.3 Interfering with the Mechanism of Action for Toxic Effects): Regarding the statement "Atropine is a competitive antagonist at muscarinic receptor sites and since it

crosses the blood-brain barrier", the Reviewer stated "Please note that the high pKa of atropine decreases its ability to cross the blood brain barrier. This is the reason high doses of atropine are generally needed to block the acute CNS signs of organophosphorus toxicity."

RESPONSE: *The statement regarding the decreased ability of atropine to cross the blood-brain barrier was not added because it does not appear to add value to the purpose of the toxicological profile for tribufos.*

COMMENT: P60, L34 to P61, L4 (Section 3.11.3 Interfering with the Mechanism of Action for Toxic Effects): The Reviewer suggested adding the following: "Limitations of the conventional antidotal therapy against organophosphorus intoxication have been reported. For example, 2-PAM is not an effective antidote against all organophosphorus compounds. In addition, 2-PAM does not cross the blood brain barrier, and, as such, it is ineffective at reversing the neurotoxic effects of organophophorus compounds. After evaluating clinical outcomes, Buckley et al. (2011) have concluded that current clinical evidence is not sufficient to indicate whether oximes are harmful or beneficial as antidotes against organophosphorus intoxication."

RESPONSE: *The statements regarding the use of oximes such as praxidoxime (2-PAM) was revised to the following: "Pralidoxime chloride (2-PAM) is a quaternary amine oxime that reverses the phosphorylation of non-aged AChE and thereby restores activity. Oximes function by nucleophilic attack on the phosphorylated enzyme; the oxime-phosphonate is then split off, leaving the regenerated enzyme. Unfortunately, the effectiveness of oximes is uncertain; however, oximes (when available) should be administered as soon as possible after exposure."*

COMMENT: P64, L12-14 (Section 3.12.2 Intermediate-Duration Exposure): Regarding the statement "The potential for tribufos to cause neurological effects has been adequately assessed for inhalation, oral, and dermal exposure routes", the Reviewer stated "This is not substantiated by the data presented in this profile." The Reviewer further stated "The data presented in this profile provide no quantitative information on the potential effects of tribufos on cognitive, sensory, or motor functions. Most assessments of neurological function seem to be restricted to cageside observations. Therefore, it seems premature to conclude that potential for tribufos to induce neurological effects has been adequately assessed."

RESPONSE: *The statement in question was revised to note that tribufos has been adequately assessed for systemic end points other than neurological effects.*

COMMENT: P65, L22-24 (Section 3.12.2 Developmental Toxicity): Regarding the statement "Additional animal studies do not appear necessary because the general population is not expected to be exposed to tribufos at oral doses in the range resulting in developmental effects in laboratory animals.", the Reviewer stated "Please note that neurobehavior (including cognitive and non-cognitive functions and structural brain integrity) were not assessed in offspring prenatally exposed to levels of organophosphorus pesticides that did not trigger overt signs of acute toxicity. Therefore, this conclusion is premature and not based on scientific data."

RESPONSE: *The following statement was added to the section: "Additional developmental toxicity studies in animals should be designed to evaluate possible tribufos-related effects on behavior and cognitive function and brain morphometry."*

COMMENT: P66, L4-5 (Section 3.12.2 Neurotoxicity): Regarding the statement "The neurotoxicity of tribufos in animals has been adequately assessed", the Reviewer stated "This conclusion is not substantiated by scientific data."

RESPONSE: *The statement in question was deleted and the following statement was added to Section 3.12.2 Developmental Toxicity): "Additional developmental toxicity studies in animals should be designed to evaluate possible tribufos-related effects on behavior and cognitive function."*

COMMENT: P66, L22-23 (Section 3.12.2 Biomarkers of Exposure and Effect): Regarding the statement "It is not likely that tribufos-specific biomarkers of effect exist.", the Reviewer stated "There is no scientific basis to justify this assertion."

RESPONSE: *The statement in question was deleted.*

COMMENT: P69, Table 4-1 (Chemical Identity of Tribufos): The Reviewer stated "In the text, Folex appears to be something other than tribufos. Please clarify."

RESPONSE: *In response to a previous comment, the following text was added to describe folex: "(formulations of folex previously used merphos, which is rapidly transformed in the environment to merphos oxide [tribufos])."*

COMMENT: P73, Table 5-2 (U.S. Companies Manufacturing Tribufos Products): Regarding the percent active ingredient in AX Tribufos, the Reviewer stated "Could the authors comment whether the remaining 30% of this commercial product – and the others in this list – is made up of pharmacologically active or inactive chemicals?"

RESPONSE: *No revision necessary. The remaining 30% are "inert" ingredients, which are typically declared confidential business information by industry.*

COMMENT: P86, L23 (Section 6.4.4 Other Environmental Media): The Reviewer stated: "Is there any information regarding potential contamination of meat with tribufos?"

RESPONSE: *No revision necessary. EPA has set tolerances for meat products (see Table 8-1). The text describes exposure from of consumption of livestock that may have been fed cotton gin-byproducts, cottonseed hulls, or cottonseed meal. However, no levels have been reported.*

COMMENT: P89, L16 (Section 6.6 Exposures of Children): Regarding the statement "No studies were identified that showed tribufos levels in mothers' milk or cord blood.", the Reviewer stated "Do you mean that there have been no studies that assessed levels of tribufos in mothers' milk or cord blood?"

RESPONSE: *The statement was revised to read as follows: "No studies were identified that assessed tribufos levels in mothers' milk or cord blood."*

COMMENT: P94, L25-27 (Section 6.8.1 Exposures of Children): Regarding the statement "Since tribufos is very rarely detected in food sources and the estimated intakes are so low, no data needs are identified at this time.", the Reviewer stated "It appears, however, that tribufos levels have not been measured in milk of lactating mothers and in maternal/fetal cord blood obtained from individuals living near or working in sites where tribufos is sprayed. This is an important piece of information needed for adequate assessment of the potential for exposure of developing fetuses/infants to tribufos."

RESPONSE: *The statement in question was revised to state the following: "Tribufos is very rarely detected in food sources and the estimated intakes are low; however, tribufos levels have not been assessed in milk of lactating mothers and in maternal/fetal cord blood obtained from individuals living near or working in sites where tribufos is sprayed. This information is needed for adequate assessment of the potential for exposure of developing fetuses/infants to tribufos."*

COMMENT: P102, L32 to P103, L1 (Section 7.3.1 Methods for Determining Biomarkers of Exposure and Effect): The Reviewer suggested adding the following text: "In addition, as described earlier, levels of RBC AChE inhibition do not always reflect the severity of organophosphorus intoxication."

RESPONSE: *The suggested addition of information regarding RBC AChE inhibition was not added because the section was revised to note that tribufos affects the central nervous system by inhibiting neural AChE.*