

Disposition of Peer Review Comments for Toxicological Profile for Hexachlorobenzene

Prepared by:

**SRC, Inc.
7502 Round Pond Road
North Syracuse, NY 13212**

Prepared for:

**Agency for Toxic Substances and Disease Registry
U.S. Public Health Service
Cassandra Smith and Henry Abadin, Co-Program Managers**

October 2012

Peer Reviewers for the second draft of the Toxicological Profile for Hexachlorobenzene were:

Eric P. Hoffman, Ph.D.
Center for Genetic Medicine Research
Children's National Medical Center
111 Michigan Ave NW
Washington, DC 20010

Lucio Costa, Ph.D.
Professor, Department of Environmental Health
University of Washington
Seattle, WA 98195

Shane Que Hee, Ph.D.
UCLA School of Public Health
Box 951772
Los Angeles, CA 90095

ATSDR would like to thank these scientists for their review of the document. When the Reviewer's suggestions were followed, or when other revisions obviated the need to respond, no further response is provided herein. Revisions that may have obviated the need to respond included sections that were rewritten, moved, or deleted. Suggestions made by the Reviewers that ATSDR decided not to follow are discussed below. In the discussion that follows, "PRP" followed by a number refers to the appropriate page of the submitted peer review summary document, "P" indicates a page number in the second draft of the profile, and "L" indicates the line number on that page. Dr. Eric Hoffman submitted comments in a separate summary document. Dr. Lucio Costa submitted embedded comments within the text of a pre-public comment draft 2 file of the 2012 toxicological profile for hexachlorobenzene. Dr. Shane Que Hee submitted comments as strike-through (suggested deletions) and underlined text (suggested additions) within an electronic file of pre-public comment draft 2 of the 2012 toxicological profile for hexachlorobenzene.

Review comments provided by Dr. Eric Hoffman:

PRP1, P8 (Some regulations...): The Reviewer stated that the reader may be confused by the statement that an adult can be exposed to up to 0.2 mg/L of hexachlorobenzene for several years without any adverse effect. The Reviewer noted that consumption of hexachlorobenzene would be 0.006 mg/kg/day based on a drinking water concentration of 0.2 mg/L and consumption of 2 L of water per day by a person weighing 70 kg. The Reviewer indicated that this dose is significantly higher than the ATSDR intermediate- and chronic-duration oral MRLs for hexachlorobenzene.

Response: The correct value (DWEL) for adult lifetime exposure to hexachlorobenzene whereby it is assumed that 100% of the exposure is via the drinking water is 0.03 mg/L, not 0.2 mg/L as stated. Based on the corrected value of 0.03 mg/L, the adult dose would be 0.0008 mg/kg/day. This value is less than one order of magnitude higher than the intermediate-duration oral MRL of 0.0001 mg/kg/day and approximately one order of magnitude higher than the chronic-duration oral MRL of 0.00007 mg/kg/day derived by ATSDR based on results from a more recent study than the one used by EPA to derive drinking water exposure limits.

PRP2, P13 (last paragraph): The Reviewer suggested deleting the phrase “as low as” from the statement “doses as low as 16 mg/kg/day”.

Response: The suggested deletion was not made because it is important to note the lowest dose level resulting in the stated effects.

PRP3, P37, L13: The Reviewer suggested moving the brief section on hexachlorobenzene and arthritis in humans from the musculoskeletal effects section to metabolic effects since the main issue is inflammation.

Response: The suggested move was not made. Although the painless arthritis of the joints is likely an inflammatory response, it occurred in the musculoskeletal system. It is considered appropriate to leave the statement in this section rather than move it to metabolic effects. The other effects in this paragraph (osteoporosis, shortening of digits of the hand) are not metabolic effects *per se*.

PRP3, P62, L24: The Reviewer asked whether any information is available regarding the purity of hexachlorobenzene in the primate studies.

Response: Purity was not reported in most of the studies. It is assumed that the purity was high in all studies. The studies in which the ovarian effects were observed at dose levels much greater than 0.01 mg/kg/day were performed using doses ≥ 4 mg/kg/day (i.e., they did not include lower doses).

PRP4, P107, L24: The Reviewer suggested deleting the boilerplate statement: **“If PBPK models for hexachlorobenzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.”**

Response: The statement in question is standard boilerplate text that is generic to all ATSDR toxicological profiles. The requested deletion was not made.

PRP4, P109, L13: The Reviewer questioned the usefulness of determining the effect of partial hepatectomy on distribution of hexachlorobenzene.

Response: The usefulness may be questionable, but it is included in the described model.

PRP5, P123, L24: The Reviewer suggested adding a sentence to boilerplate text in Section 3.7 (Children's Susceptibility) to indicate that increased susceptibility to children involves the central nervous system.

Response: The requested addition was not made. It does not appear necessary to place a focus on the developing central nervous system in the general statement that is made in the boilerplate.

All other comments provided by Dr. Hoffman were addressed as suggested.

Review comments provided by Dr. Lucio Costa:

P1 (Public Health Statement for Hexachlorobenzene), Comment A1: The Reviewer stated that the Public Health Statement should note that children are more sensitive than adults to hexachlorobenzene exposures.

Response: It is stated in the section titled "Children and Hexachlorobenzene" that children appeared to be especially sensitive to the effects of hexachlorobenzene in the Turkish grain poisoning epidemic. It is also stated that young animals exposed to hexachlorobenzene before and soon after birth are especially sensitive.

P3, Comment A7: The reviewer inquired if hexachlorobenzene had any direct effect on environmental issues (e.g., ozone depletion, global warming).

Response: Although hexachlorobenzene is a persistent organic pollutant (POP), its effect on the ozone is negligible since the amounts that may make it into the stratosphere are extremely low in comparison to highly volatile chlorinated substances such as chlorofluorocarbons (CFCs). Wet deposition, combined with the low levels of hexachlorobenzene currently in the atmosphere, indicate that it is not likely to trap significant quantities of thermal radiation from the Earth's surface, nor is it expected to reach upper layers of the atmosphere. Therefore, it is not likely to be associated with global warming.

P4, Comment A9: The Reviewer stated that age dependency was not mentioned in the section titled "Introduction to hexachlorobenzene health effects."

Response: The issue of age dependency is addressed in the section titled "Children and Hexachlorobenzene".

P6, Comments A12 and A13: The Reviewer suggested giving a brief description of the Turkish grain poisoning epidemic.

Response: The details of this epidemic are found in Chapter 3. The various sections of the Public Health Statement are intended to present only concise summary information. The requested addition of descriptive details of the Turkish grain poisoning epidemic was not made.

P6, Comment A15: The Reviewer suggested referencing the statement that some technical-grade pesticides or solvents contain hexachlorobenzene as an impurity and asked whether testing for hexachlorobenzene in products is required.

Response: The statement was revised as follows: "In the past, some technical-grade pesticides or solvents when produced were found to contain trace amounts of hexachlorobenzene as an impurity. However, levels of hexachlorobenzene would be expected to be much lower than those causing health

problems.” Recent references to products containing hexachlorobenzene as a byproduct or contaminant were not located.

P7, Comment A17: The Reviewer requested the addition of a list of sites where breast milk samples could be tested. The Reviewer further asked whether there is evidence of hexachlorobenzene in infant formula, what proportion of lactating women have elevated levels of hexachlorobenzene in their milk, and whether there are any known risk factors.

Response: The requested additional information is beyond the scope of this section of the Public Health Statement. Quantitative information is presented in Chapters 3–7.

P9, Comment A18: The Reviewer suggested adding a section on testing sites/services to the “Additional information from ATSDR” section of the Public Health Statement.

Response: ATSDR will consider the request for additional information on testing sites; if deemed appropriate, the additional information will be included in future drafts of the Toxicological Profile for Hexachlorobenzene.

P10, Comment A21: The reviewer noted that the estimates of hexachlorobenzene produced in chlorinated solvents are old and inquired if newer studies were available.

Response: No additional data were located.

P10, Comment A22: The reviewer asked if the uses of hexachlorobenzene in the production of pyrotechnics, ordinances and synthetic rubber were current.

Response: The sentence was written in the past tense (e.g., former uses).

P14, Comment A23: The Reviewer suggested moving details regarding the Turkish poisoning epidemic in Section 2.2 to the Public Health Statement.

Response: The details are beyond the scope of the Public Health Statement. The requested move was not made.

P19, Comment A24: The Reviewer requested that the statement regarding a preliminary report of infants from Flix, Spain, that found an association between high hexachlorobenzene levels in milk and blood and impaired development of motor skills should be expanded with descriptive details.

Response: The statement was slightly revised. However, this section is intended as a brief summary of the toxicity data for hexachlorobenzene. The quantitative data are presented in Chapter 3.

P20, Comment A25; P21, Comment A26: The Reviewer stated that the intermediate- and chronic-duration oral Minimal Risk Levels (MRLs) for hexachlorobenzene should be split by age ranges because there are clear associations with developmental age.

Response: MRLs are derived using the highest no-effect level and/or most sensitive effect level for the critical effect. Uncertainty factors are included to account for particularly sensitive populations. Therefore, the MRL is intended to be protective of possible age-related differences that have not necessarily been demonstrated in humans or animals. The requested division by age group was not made.

P22, Comment A27: The Reviewer asked why the chronic-duration oral MRL is based on the lowest risk population.

Response: The critical effect was selected based on the lowest effect level from the available database of toxicity data. In this case, hepatic effects were observed in first-generation male offspring at a dose level (0.022 mg/kg/day) that was lower than the lowest dose for any other effect. Uncertainty factors account for possible age-, gender-, and species-related differences in susceptibility.

P24, Comment A28: The Reviewer asked why the health effects data presented in Chapter 3 are stratified by risk group.

Response: ATSDR toxicological profile convention is to present health effects data in terms of acute-, intermediate-, and chronic-duration exposure. Within these parameters and for each health effect, age-related information is captured. Additional stratification is not considered necessary.

P33, Comment A29: The Reviewer questioned the order of exposure route used in the profile and stated that for hexachlorobenzene, oral exposure should be presented first because it represents the highest risk mode of exposure.

Response: The order (inhalation, oral, dermal) is consistent between toxicological profiles. This way, the reader can more readily access a particular section of the profile. The suggested change was not made.

P33, Comment A31: The Reviewer stated that one should be able to use the reported levels of hexachlorobenzene in the breast milk to roughly calculate hexachlorobenzene enrichment in the breast milk relative to blood levels.

Response: One could make rough calculations; however, the important point is that levels were higher in blood of infants (particularly breast-fed infants) than maternal blood.

P34, Comment A32: The Reviewer stated that the enhancement of hexachlorobenzene toxicity by use of an oil vehicle in oral animal studies is important in terms of monitoring human dose because different types of exposure (factory likely in crystalline form; breast milk in lipid-associated form) would dictate relative toxicity. The Reviewer indicated that since most human exposure would likely be to lipid-associated hexachlorobenzene via the diet, this point should be carefully described in the Public Health Statement.

Response: A statement does not appear necessary because human concern is most likely exposure via lipid-associated ingestion.

P95, Comment 37: The Reviewer stated that it is important to point out that infant formula has less hexachlorobenzene and asked whether new popular lipid additives to formula have been tested for hexachlorobenzene.

Response: No information was located regarding levels of hexachlorobenzene in infant formulas. The finding of lower levels in bottle-fed infants compared to breast-fed infants does not necessarily translate to exposure via infant formula because both groups of infants were exposed in utero as well.

P117, Comment 40: The Reviewer stated that it would be helpful to have an opening paragraph in Section 3.5.2 (Mechanisms of Toxicity) describing current thinking regarding possible mechanisms of toxicity.

Response: An introductory statement was added to inform the reader of available information regarding various end points of hexachlorobenzene toxicity. The bulk of mechanistic data is related to porphyria

and associated effects. The detailed portion of Section 3.5.2 was rearranged to group discussion according to end point where possible.

P124, Comment A42: The Reviewer stated that the finding of ultrastructural lesions consistent with lipid peroxidation in mitochondria from the ovaries of hexachlorobenzene-treated monkeys is a mechanism of toxicity that may be more broadly important than the heme biosynthesis pathway and that this information should be included in the Public Health Statement as well as in an introductory overview statement in Section 3.5.2.

Response: The Public Health Statement is not intended to include information regarding possible mechanisms of toxicity. The information regarding mechanisms of hexachlorobenzene-induced effects on monkey ovaries was added to Section 3.5.2.

P162, Comment A43. The reviewer commented that given the persistence and environmental dissemination of hexachlorobenzene, there should be a paragraph on production in other countries.

Response: Production and use is expected to be low even in developing countries where this substance is not banned; however, data on production numbers are difficult to obtain and are likely to be unpredictable for any given year.

P166, Comment A44: The reviewer commented regarding Figure 6-1 that the legend was difficult to read and requested the numbers be added to the states.

Response: The legend was expanded, but adding the numbers to the states resulted in a legend that was even more difficult to read.

All other comments provided by Dr. Costa were addressed as suggested.

Review comments provided by Dr. Shane Que Hee:

P6, Paragraph 2: The Reviewer suggested adding the word “mother’s” to the statement regarding levels of hexachlorobenzene in milk of breast-fed babies.

Response: The suggested addition is redundant as breast-fed babies were assumed to have nursed from their mothers.

P7, Paragraph 1: The Reviewer suggested adding the following sentence to the Public Health Statement on measurement of hexachlorobenzene in blood, urine, and feces: “The production of persistent purple urine is diagnostic.”

Response: The sentence was not added. The public should consult a physician when any discolored urine is produced.

P8, Paragraph 3: The Reviewer suggested adding the sentence “Workplace air level recommendations are also available from the American Conference of Governmental Industrial Hygienists” to the section titled “Check for regulation updates.”

Response: ACGIH recommendations are covered in Chapter 8. The requested addition was not made.

P13, Last paragraph: The Reviewer added the following sentence to the section titled “Reproductive effects:” “For example, endometriosis (tissue that normally lines the inside of the uterus — the

endometrium — grows outside the uterus, most commonly involving the ovaries, bowel or the tissue lining the pelvis) can cause fertility problems; endometriosis has been correlated with serum hexachlorobenzene (Cooney et al 2010).”

Response: The requested addition was not retained. The study of Cooney et al. (2010) provides suggestive evidence for a possible association between serum hexachlorobenzene and endometriosis. However, the evidence is no more convincing than that from other investigators.

P23, End of Section: The Reviewer suggested adding the following statement: “In a review, Aylward et al (2010), proposed that serum lipid adjusted hexachlorobenzene concentrations from 16 to 250 ng/g lipid were associated with non-cancer-based exposure guidance values from various American Government agencies.”

Response: This section is reserved for discussion of ATSDR-derived MRLs. The proposal of Aylward et al. (2010) does not include established guidance or regulatory values; therefore, the proposed values are not included in Chapter 8.

P28 and other sections: The Reviewer changed the spelling of the Spanish island of Menorca to “Minorca”.

Response: The spelling “Menorca” was retained because it is the correct Spanish spelling for the island name. Technically, both spellings are correct.

P63, Top of page: The Reviewer suggested adding a statement to the summary of the study of Cooney et al. (2010) to note the monitoring of other chlorinated pesticides (aldrin, trans-nonachlor, p,p'-DDE, and beta-BHC) in the study and the potential for increased incidences of endometriosis to have been caused by exposure to trans-nonachlor. The Reviewer also suggested deleting the study limitation of lack of quantification of hexachlorobenzene exposure levels.

Response: The statement regarding the monitoring of other chlorinated pesticides (aldrin, mirex, trans-nonachlor, p,p'-DDE, and β -BHC) was not included in the suggested manner. Instead, the groupings according to chemical structure were added as presented in the study report of Cooney et al. (2010). This section was revised to more accurately capture the numbers of women in each study group who were diagnosed with endometriosis and those who did not have the condition. The study limitations were retained because they are relevant and correct. Although the odds ratio could have been influenced by trans-nonachlor or other factors, when grouped according to chemical structure, a significant odd ratio was found only for the group of aromatic fungicides (including hexachlorobenzene); there was no significant odds ratio for the grouping of cyclodiene insecticides (included aldrin, mirex, and trans-nonachlor). Therefore, the statement regarding the possibility of the increased incidence of endometriosis being attributable to trans-nonachlor was not retained.

P115-118 (Section 3.5.1): The Reviewer suggested several changes to this section.

Response: This section was reduced to a simple statement regarding possible mechanisms involved in the pharmacokinetics of hexachlorobenzene. The reader is also referred to Section 3.4 for detailed toxicokinetic information. This change was made in part because Section 3.5.1 contained information already presented in Section 3.4. The equation of Schlummer et al. (1998) erroneously identified the units for net absorption in Table 5 as percent net absorption when it should be net fractional absorption. This was corrected in Section 3.4 and the associated text was revised to better reflect the results of the study. The discussion of the “fat flush” theory was deleted.

P142, last sentence, P147, end of first paragraph: The Reviewer suggested adding a sentence to note that mechanistic commonalities of the dioxin-like behavior of hexachlorobenzene with that of tetrachlorodibenzo-*p*-dioxin (TCDD) and other analogs should be further explored (Mundy et al., 2010, 2012) and the effects of hexachlorobenzene on apoptosis should be investigated to develop the results of Luan et al. (2011).

Response: The suggested addition was not made to P142 because this section is intended only to inform the reader of existing data or lack thereof. The suggested addition to P147 was revised to note the need for additional studies that assess possible mechanisms of action for hexachlorobenzene toxicity without specifically calling out studies of commonalities between hexachlorobenzene and TCDD or *in vitro* indications that hexachlorobenzene affects apoptosis.

P244, ACGIH carcinogenicity classification: The Reviewer suggested adding the value of 0.002 mg/m³ to the column “Information” in Table 8-1.

Response: The suggested addition was not made because the value of 0.002 mg/m³ is a Threshold Limit Value (TLV) for a noncancer end point. This value appears in the appropriate section of Table 8-1.

All other comments provided by Dr. Shane Qee Hee were addressed as suggested.