DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR 3,3'-DICHLOROBENZIDINE

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

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Peer reviewers for the third pre-public draft of the Toxicological Profile for 3,3'-Dichlorobenzidine were:

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

Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

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

COMMENT: Disagree with non-cancer effects in humans attributed to Gerard and Gerad other than dermal and made comments/tracked changes throughout the text.

RESPONSE: *ATSDR agrees with the reviewer's comment on the Gerarde and Gerarde study. The study was re-examined and statements citing this study with regard to neurological, gastrointestinal and respiratory effects in workers were deleted. The study does not provide sufficient evidence to make statements relating the workers' 3,3'-dichlorobenzidine exposure to these effects. The reviewer made several in-text edits noting points where these statements are made, and those specific comments are addressed under Annotated Comments below.*

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

COMMENT: Cancer effects in animals are possible in humans and are reasonably presented. Why because of similarity to benzidine

RESPONSE: *No revisions were suggested.*

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

COMMENT: Multiple comments in text on ways to tighten up the exposure descriptions/use and likelihood of exposure.

RESPONSE: *The reviewer made multiple in-text comments relating to exposure and are listed below under Annotated Comments. Comment-specific responses are also provided.*

Chapter 2. Health Effects

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

COMMENT: Have suggested changes in description/interpretation of epidemiologic studies.

RESPONSE: *The reviewer made multiple in-text comments relating to the human epidemiologic studies in the Profile. These specific comments and corresponding responses are listed below in Annotated Comments.*

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

COMMENT: Studies that exist were addressed. Multiple comments in text on how better to describe the results.

RESPONSE: *The reviewer made multiple comments regarding the presentation of study findings. These specific comments and corresponding responses are listed below in Annotated Comments.*

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

COMMENT: In my perspective as an epidemiologist, review was adequate.

RESPONSE: *No revisions were suggested.*

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

COMMENT: In my perspective as an epidemiologist, animal species were appropriate.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes adequate attention to dose-response and lack of such data for human studies.

RESPONSE: *No revisions were suggested.*

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

COMMENT: I added one ATSDR reference that is peripherally related.

RESPONSE: *The reviewer recommended the inclusion of an ATSDR document "Self-Testing for Hematuria Among a Population of Community Residents, Workers, and Family Members with Potential Exposure to Bladder Carcinogens at Three Locations in Michigan" published December 1999, publication number PB99-159840. The study was reviewed and the information pertaining to 3,3'-dichlorobenzidine is from the 1996 ATSDR assessment of the Bofors-Nobel factory in Michigan. This*

study was not added as a reference, as it was confirmed to contain the same information as the ATSDR (1996) study already cited in the profile.

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

COMMENT: Not aware of any

RESPONSE: *No revisions were suggested.*

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

COMMENT: In my perspective as an epidemiologist, NOAELs and LOAELs were appropriate.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

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

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: Not that I am aware

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, adducts and chemical itself

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, adducts and chemical itself

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

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

COMMENT: Not that I am aware

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

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

COMMENT: Many comments about this in text. Information was incomplete and not consistent across sections. I am not aware of additional references.

RESPONSE: *The reviewer made multiple in-text comments and edits. These comments and corresponding responses are addressed below in Annotated Comments. Many pieces of information were added and deleted as recommended by the reviewer.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound

information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: NO because not consistent in what types of industries DCB was used or is still used.

RESPONSE: *In the text, the reviewer made multiple comments regarding points in the text where it is unclear in which industries 3,3'-dichlorobenzidine is used and the state of its current use. These comments and corresponding responses are addressed below in the Annotated Comments. 3,3'-Dichlorobenzidine is primarily used in dyes and pigments, which have the possibility of being used in other products. Additionally, trace amounts of 3,3'-dichlorobenzidine have been measured in cosmetic products, however it is unknown if the chemical is used in the production of these products.*

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

COMMENT: Yes, not aware of more references.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, not aware of more references. Discussion of quality adequate

RESPONSE: *No revisions were suggested.*

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

COMMENT: Same answer as 2 above.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

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

COMMENT: Rosenman 2004 study has been updated and submitted for publication.

RESPONSE: *Reference to the update of this study has been added to Section 6.3 Ongoing Studies. The update does not appear to be published yet and thus not included in the Profile.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: NO but did not adequately describe OSHA and NIOSH positions

RESPONSE: *The reviewer's suggested edits to Table 7-1 are detailed further under Annotated Comments below. These edits for Permissible Exposure Limits (OSHA) and Recommended Exposure Limits (NIOSH) guidelines were added. While no PELs nor RELs are established, the reviewer recommended adding guidance provided by OSHA and NIOSH in relation to these limits and ATSDR agreed with these inclusions.*

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

COMMENT: NO

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

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

COMMENT: Agree.

RESPONSE: *No revisions were suggested.*

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

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

COMMENT: Not applicable.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Does not need to be further addressed.

RESPONSE: *No revisions were suggested.*

Appendices

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

COMMENT: Modified some of the definitions.

RESPONSE: *Following review of the comments on the boilerplate text, ATSDR did not incorporate the edits. The boilerplate comments are collected for later review, because boilerplate changes are made to all profiles after a collective review.*

Annotated Comments on the Profile

COMMENT: IARC 2B possible not known human carcinogen 1987

RESPONSE: *This comment refers to the first paragraph in Section 1.1 (Overview and U.S. Exposures). Text originally states, "In the United States, manufacturing use has significantly decreased since its classification as a known carcinogen…" however, this was corrected to state "classification as a possible human carcinogen."*

COMMENT: Adducts on hemoglobin/lymphocytes mean measure of exposure not necessarily sensitive target

RESPONSE: *This comment refers to Section 1.2 (Summary of Health Effects). The text originally states "**Hematological Effects.** While cell counts were normal in the single study identified that examined this endpoint, adducts on hemoglobin and lymphocytes were observed with a dose response relationship in other studies. Refer to Figure 1-2 for all sensitive targets." Following further review, ATSDR agrees with the reviewer that adducts DNA adducts in lymphocytes and hemoglobin adducts should be used as a biomarker of exposure and not an effect in the context of the data presented in the Profile. The hematological effects paragraph in Section 1.2 the reviewer refers to was deleted. The Profile was revised throughout to no longer state that hemoglobin adducts are a toxic effect following 3,3'-dichlorobenidine exposure. Hemoglobin adducts as a biomarker of exposure is described in Chapter 3 of the profile. Additionally, the profile no longer states that DNA adducts in lymphocytes are a biomarker of effect nor a toxicological effect of 3,3'-dichlorobenzidine.*

COMMENT: This is a very poor summary of the study. "Only one case of bladder cancer was identified among the workers who were exposed to dichlorobenzidine only. However, an increased risk for lymphohematopoietic cancer was found among these dichlorobenzidine only workers (SMR 6.62 (95% CI 1.37–19.36))."

RESPONSE: *This comment refers to the paragraph about cancer in section 1.2 (Summary of Health Effects). The text originally states "The sixth, Rosenman and Reilly (2004) identified 22 bladder cancer cases among a cohort of 538 workers exposed to benzidine and/or 3,3'-dichlorobenzidine from a chemical manufacturing plant. Increased standardized mortality ratios were found for all cancers together, as well as bladder cancer and lymphohematopoietic cancer separately." The reviewer's comment is quoting directly from the Rosenman and Reilly (2004) abstract. ATSDR reviewed the summary of the Rosenman and Reilly (2004) study and made edits to clarify that only one case of bladder cancer was identified among workers exposed to 3,3'-dichlorobenzidine and the study found an increased risk for lymphohematopoietic cancer. The text now states "The sixth study, Rosenman and Reilly (2004), identified one case of bladder cancer among workers exposed only to 3,3'-dichlorobenzidine in a chemical manufacturing plant. Among these same workers, an increased risk for lymphohematopoietic cancer was identified (standardized mortality ratio=6.62; 95% confidence interval 1.37-19.36)."*

COMMENT: Years worked is a surrogate for quantitative data

RESPONSE: *This comment refers to the statement in the paragraph about cancer in section 1.2 (Summary of Health Effects) that discusses the limitations of the Rosenman and Reilly (2004) study. The text originally stated, "A limitation of this study is lack of quantitative exposure data for the workers." ATSDR revised this sentence to clarify that the limitation of the studies is lack of quantitative exposure data for the workers as only the number of years worked was provided. The text was updated to say "A limitation of this study is the lack of quantitative exposure data for the workers as only the number of years worked was provided."*

COMMENT: I doubt this is true. The cancer results may have been highlighted but for example Rosenman and Reilly looked at all causes of death

RESPONSE: *This comment refers to the statement in Section 2.1 (Introduction) that says, "The small number of available observational epidemiology studies primarily examined cancer endpoint." The six epidemiological studies (summarized in Table 2-1) do primarily examine cancer as the endpoint of interest. The stated purpose of the Rosenman and Reilly study was to examine bladder cancer cases among 3,3'-dichlorobenzidine exposed workers which included examining other causes of death among the workers, as shown in Table II in the study. Therefore, ATSDR has not made edits in response to this comment.*

COMMENT: Don't know what lack of follow up means.

RESPONSE: *This comment refers to the bullet for cancer targets in Section 2.1 (Introduction) that states "Cancer: Human evidence for cancer are suggestive at best. The epidemiological data are limited by*

sample size, lack of follow-up, and limited to no exposure data. Several organs in animals were impacted by cancer." This phrase was edited to include "lack of participant follow-up."

COMMENT: A major limitation not included is co - exposure to other carcinogens

RESPONSE: *This comment refers to the bullet for cancer targets in Section 2.1 (Introduction) that states "Cancer: Human evidence for cancer are suggestive at best. The epidemiological data are limited by sample size, lack of follow-up, and limited to no exposure data. Several organs in animals were impacted by cancer." Chapter 2 was revised as follows to include this limitation (co-exposure to other carcinogens):*

> *"**Cancer.** Human evidence for cancer are suggestive at best. The epidemiological data are limited by sample size, lack of participant follow-up, limited to no exposure data, and co-exposure to other carcinogens. Several organs in animals were impacted by cancer and tumors were found in multiple species."*

COMMENT: At least two of the human epidemiological studies had non cancer mortality data

RESPONSE: *This comment refers to Figure 2-1 in section 2.1. The Gerarde and Gerarde (1974) study did not relate occupational death to 3,3'-dichlorobenzidine exposure and was therefore not included for human health effects in Chapter 2.2 Death. The Rosenman and Reilly (2004) study examined mortality reported in Social Security data to identify cancer cases. The study reports the causes of death of all employees but there is no analysis on whether 3,3'-dichlorobenzidine contributed to these causes, therefore this was not included as a study examining the death endpoint. No edits were made in response to this comment.*

COMMENT: Control is specified Connecticut rates

RESPONSE: *This comment refers to the Ouellet-Hellstrom and Rench 1996 entry in Table 2-1 in Section 2.1. The entry originally stated that controls were not specified. Table 2-1 was revised and the entry for the Ouellet-Hellstrom and Rench (1996) study was edited to include the control used as follows:*

> *"Control: Cancer incidence rates from State of Connecticut were used to generate SIR values"*

COMMENT: Same comment as above

RESPONSE: *This comment refers to the Rosenman and Reilly 2004 entry in Table 2-1 in Section 2.1. The entry originally stated that controls were not specified. Table 2-1 was revised and the entry for the Rosenman and Reilly (2004) study was edited to include the control used as follows:*

> *"Control: US general population rates used to generate SMR values. Michigan cancer rates used to generate SIR values"*

COMMENT: Provided relative estimates of exposure to arylamines

RESPONSE: *This comment refers to the Ouellet-Hellstrom and Rench (1996) entry in Table 2-1 in Section 2.1. The entry originally stated that exposure levels were not specified. The description of the exposure level of the Ouellet-Hellstrom and Rench (1996) study in Table 2-1 to was revised as follows:*

> *"Exposure Level: Scoring system of 0 to 5 based on intensity and frequency of exposure to arylamines (3,3'-dichlorobenzidine, o-dianisidine, o-tolidine, o-toluidine, o-chloroaniline)."*

COMMENT: Left out the incidence results

RESPONSE: *This comment refers to the Rosenman and Reilly 2004 entry in Table 2-1 in Section 2.1. The original text says:*

> *"Among those workers exposed only to 3,3'-dichlorobenzidine, one case of bladder cancer with an SMR 8.34 95%CI: 1.72-24.38*
>
> *3 cases of lymphohematopoietic cancer among benzidine and/or 3,3'-dicholorbenzidine workers with an SMR 2.84 95% CI: 4.30-10.4*
>
> *SMR 6.62 95%CI: 1.37-19.36 for lymphohematopoietic cancer specifically among 3,3'-diclorobenzenidine only exposed workers"*

The standardized incidence ratio (SIR) for bladder cancer, as reported in the Rosenman and Reilly (2004) study was added to Table 2-1, outcomes information was revised as follows:

> *"Among deceased workers exposed to benzidine and/or 3,3'-dichlorobenzidine, 3 cases of bladder cancer with a SMR 8.34, 95% CI: 1.72-24.38. SIR for bladder cancer: 6.85, 95% CI 4.3-10.4)*
>
> *Among same group, 6 cases of lymphohematopoietic cancer with an SMR 2.84. 95% CI: 1.04-6.18*
>
> *SMR 6.62. 95% CI: 1.37-19.36 for lymphohematopoietic cancer among workers exposed to 3,3'-dichlorobenzidine only"*

COMMENT: Misleading this is not the SMR for the one bladder cancer with exposure to dichlorobenzidine

RESPONSE: *This comment refers to the Rosenman and Reilly (2004) entry in Table 2-1 in Section 2.1. The study was reviewed, and the table was revised, please see response to the comment above for revisions made in text in response to this comment.*

COMMENT: Totally inappropriate. There is a statement about the common reasons workers came to the company clinic. The conditions listed were those commonly seen in such a clinic. There was no data no suggestion work-related

RESPONSE: *This comment refers to the first paragraph in section 2.4 (Respiratory). The original text states "In an occupational study of workers handling 3,3'-dichlorobenzidine dihydrochloride, upper respiratory infection and sore throat were listed among several principal reasons for visits to the*

company medical clinic (Gerarde and Gerarde 1974). However, effects could not be conclusively linked to 3,3'-dichlorobenzidine exposure as workers were exposed to other agents at the same by inhalation and dermal routes." After further review of the Gerarde and Gerarde (1974) study, several sections in the text were deleted regarding statements of respiratory, gastrointestinal, and neurological effects in workers. In response to this specific comment, discussion of any respiratory effects from Gerarde and Gerarde (1974) were excluded from Section 2.4 Respiratory.

COMMENT: Same comment as under respiratory.

RESPONSE: *This comment refers to the text in section 2.6 (Gastrointestinal). The original text says "Gastrointestinal upset was reported by workers who handle, and likely inhaled, 3,3'-dichlorobenzidine dihydrochloride (dihydro salt of 3,3'-dichlorobenzidine) (Gerarde and Gerarde 1974). However, no conclusive evidence indicated that gastrointestinal effects, and other employee reported symptoms, resulted only from inhalation of 3,3'-dichlorobenzidine dihydrochloride." After further review of the Gerarde and Gerarde (1974) study, several sections in the text were deleted regarding statements of respiratory, gastrointestinal, and neurological effects in workers. In response to this specific comment, discussion of any gastrointestinal effects from Gerarde and Gerarde (1974) were excluded from Section 2.6 Gastrointestinal.*

COMMENT: Same as respiratory.

RESPONSE: *This comment refers to the first paragraph in section 2.15 (Neurological). The original text states "The only relevant information regarding neurological effects in humans exposed to 3,3'-dichlorobenzidine via inhalation was found in an early study which reported that headache and dizziness were among several principal reasons chemical manufacturing plant employees working with 3,3'-dichlorobenzidine visited the company medical clinic (Gerarde and Gerarde 1974). However, there is no conclusive evidence that these symptoms were caused specifically by 3,3'-dichlorobenzidine since the employees were exposed to other chemicals as well. No further information was provided." After further review of the Gerarde and Gerarde (1974) study, several sections in the text were deleted regarding statements of respiratory, gastrointestinal, and neurological effects in workers. In response to this specific comment, discussion of any human neurological effects from Gerarde and Gerarde (1974) were excluded from Section 2.15 Neurological.*

COMMENT: More than no association they reported no bladder cancers

RESPONSE: *This comment refers to the first paragraph in section 2.19 (Cancer). The original text states "Six epidemiological studies were identified. Three of the six epidemiological studies identified reported no association between exposure to 3,3'-dichlorobenzidine and increase in incidence or death due to bladder cancer (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975). These three studies had limited power to detect an exposure-related effect due to small sample size, and short follow-up time, no control group, or co-exposure to other chemicals." Gerarde and Gerarde (1974) reported that based on the literature, there is a positive association between occupation and bladder cancer. However, it is unable to establish that exposure to 3,3'-dichlorobenzidine specifically is associated with increased incidence of bladder cancer.*

MacIntyre (1975) and Gadian (1975) reported no bladder cancer among workers exposed to 3,3'-dichlorobenzidine, however bladder cancer was seen among workers exposed to a mixture of benzidine and 3,3'-dichlorobenzidine.

Text was edited to further clarify the difference in the findings of these studies, as follows:

> *"Six epidemiological studies were identified. One study reported no association between exposure to 3,3'-dichlorobenzidine and increase in incidence or death due to bladder cancer (Gerarde and Gerarde 1974). Two studies identified no cases of bladder cancer among workers in occupational settings where exposure to 3,3'-dichlorobenzidine was exclusive (Gadian 1975; MacIntyre 1975), however cases of bladder cancer were reported among workers exposed to a mixture of benzidine and 3,3'-dichlorobenzidine (Gadian 1975). These three studies had limited power to detect an exposure-related effect due to small sample size, short follow-up time, no control group, or co-exposure to other chemicals. While both Myslak et al. (1991) and Ouellet-Hellstrom and Rench (1996) reported an increase in bladder tumors in workers exposed to either benzidine-based azo dyes (Myslak 1996) or arylamines (Ouellet-Hellstrom and Rench 1996), a direct attribution to 3,3'-dichlorobenzidine cannot be made."*

COMMENT: These two issues don't matter if there are no bladder cancers

RESPONSE: *This comment refers to the statement "These three studies had limited power to detect an exposure-related effect due to small sample size, short follow-up time, no control group, or co-exposure to other chemicals" in the first paragraph of section 2.19 (Cancer), which discusses Gadian 1975, Gerarde and Gerarde 1974, and MacIntyre 1975. The two issues in question are lack of a control group and co-exposure to other chemicals. See response to previous comment for edits to this section.*

COMMENT: No where in the report does it cite commonly used in consumer products

RESPONSE: *This comment refers to the first paragraph in Section 4.1 (Chemical Identity). The sentence states "3,3'-Dichlorobenzidine (and its salts) was previously used in the manufacture of dyes in the United States, but no longer sees common use in consumer products." ATSDR agreed with the reviewer's comments and made the following edits:*

> *"3,3'-Dichlorobenzidine (and its salts) was previously used in the manufacture of dyes in the United States."*

COMMENT: Data cited in 5.2 does not support decreased use of 3,3 dichlorobenzidine in the US. And I have modified the bullet. If the authors can find more data on historical and current use that data should be added and perhaps modify what I have written. If not than way I rewrote is more factual.

RESPONSE: *This comment refers to the fourth bullet in section 5.1 (Overview), which originally stated "Manufacturing use has decreased significantly since 1986 in the United States, and occupational exposure is less likely to occur. The bullet in Section 5.1 was edited to clarify as follows:*

"The manufacturing of 3,3'-dichlorobenzidine has markedly decreased since 1986 in the United States, but large quantities are still imported into the United States and occupational exposure remains the major source of exposure."

COMMENT: Not clear from this paragraph how the ~6 million pounds of dichlorobenzidine currently imported each year into US is used

RESPONSE: *This comment refers to section 5.2.3 (Use). Section 5.2.2. Import/Exposure states that in 2018, 5,773,111 pounds were imported from China and India, cited from USITC. USITC only provides data on the pounds imported and not any further details to state or make assumptions on its use. No edits were made in response to this comment.*

COMMENT: No mention of polybenzimidazole (PBI), which is mention in Section 5.6

RESPONSE: *This comment refers to section 5.2.3 (Use). Section 5.6 was edited to include that 3,3'-dichlorobenzidine is used in the production of PBI. The following edit was made:*

"The greatest chance of exposure to 3,3'-dichlorobenzidine by the general public is from its persistence in the environment attributed to improper land disposal of compounds. The importance of this exposure route can only be evaluated on a site-by-site basis but the potential for nonindustrial exposure via air, soil, or water is expected to be negligible. 3,3'-Dichlorobenzidine is no longer used to manufacture soluble dyes in the United States (CPMA 1998). Previously benzidine and its congeners such as 3,3'-dichlorobenzidine were likely to only be found in the vicinity of pigment plants (EPA 1980; EPA 1983a; Shriner et al. 1978) where wastes may escape or be discharged. 3,3'-Dichlorobenzidine was also be found in locations where it was used to formulate other products such as rubber and plastic (PubChem 2019) or to produce polybenzimidazole (PBI) (Celanese 1985)."

COMMENT: In Section 5.5, waste water of metal finishing and oil field operations have dichlorobenzidine. Nothing here about dichlorobenzene related to either.

RESPONSE: *This comment refers to section 5.2.3 (Use). The text was edited to include the possible use in metal finish and oil operations. Section 5.2.3 Use was edited as follows:*

"3,3'-Dichlorobenzidine had been primarily used in the production of yellow, and some red and orange pigments for the textile, paint, printing inks, paper, rubber, plastic, and pharmaceutical industries (PubChem 2019; EPA 2010). Currently, use of 3,3'-dichlorobenzidine in the production of dyes has stopped, as dye production in the U.S. has largely ceased and has shifted abroad (Dapson 2009). 3,3'-Dichlorobenzidine has also been used as a curing agent for isocyanate-containing polymers and solid urethane plastics, and as a compounding ingredient for rubber and plastics (PubChem 2019). The chemical can also be used to produce polybenzimidazole (Celanese 1985) or be used as a color test for the detection of gold (PubChem 2019). 3,3'-Dichlorobenzidine has been detected in the wastewater of metal finishing operation facilities and oilfield operations (See Section 5.5), suggesting they are used in these industries,

but no specific information on its use is available. Additionally, the chemical is likely used in steam electric power generation based on its EPA regulatory limitation (EPA 2018a)."

COMMENT: Word significance typically reserved for when talking about statistical significance

RESPONSE: *This comment refers to the sentence in the first paragraph of section 5.6 (General Population Expsoure), which originally states "The significance of this exposure route can only be evaluated on a site-by-site basis but the potential for nonindustrial exposure via air, soil, or water is expected to be negligible." The sentence was edited to exclude the word "significance" as follows:*

"The importance of this exposure route can only be evaluated on a site-by-site basis but the potential for nonindustrial exposure via air, soil, or water is expected to be negligible."

COMMENT: Still used or were used. Not mentioned 5.2.3.

RESPONSE: *This comment refers to the sentence in the second paragraph of section 5.6 (General Population Exposure), which states "3,3'-Dichlorobenzidine-based pigments are normally used in printing ink applications; their use in paints is rare and, thus, its presence in present-day pressurized paint spray would not be expected (CPMA 1998)." The wording was edited to state "Dichlorobenzidine-based pigment have been used in..." These uses are currently included in Section 5.2.3: "3,3'-Dichlorobenzidine had been primarily used in the production of yellow, and some red and orange pigments for the textile, paint, printing inks, paper, rubber, plastic, and pharmaceutical industries (HSDB 2019; EPA 2010)."*

COMMENT: Are these current or historical. Not mentioned in use section 5.2.3.

RESPONSE: *The reviewer is highlighting the following statement in section 5.6 (General Population Exposure): "Occupational exposure to 3,3'-dichlorobenzidine is most likely to occur in the synthesis of pigments, the compounding of lead iodide, and the garment, leather, printing, paper, and homecraft industries where benzidine-based pigments are used." The stated use here is "benzidine-based pigment," and this statement highlights where occupational exposure may occur due to use of these pigments. No further edits were made in response to this comment.*

COMMENT: No data in this report that may be in paint?

RESPONSE: *This comment refers to the sentence in the sixth paragraph in section 5.6 (General Population Exposure) that states "Young children may be exposed to 3,3'-dichlorobenzidine by ingesting paint chip debris, colorful objects or paints, and soil if the material contains the chemical." 3,3'-Dichlorobenzidine has been measured in wastewater from paint and ink formulation operations as stated in section 5.3.2 Water: "and up to 10 ppb in paint and ink formulation operations (EPA 1983a)." Additionally, it is quoted throughout the profile that 3,3'-dichlorobenzidine had been used in paint, lacquers and enamels in the past. Its use in paints now is less likely but 3,3'-dichlorobenzidine paints may still remain on surfaces, and it would be reasonable to assume that paint chip debris could be a possible source of exposure, especially for children. The Myslak et al. (1991) study, as reported in Table*

2-1, demonstrates occupational exposure to 3,3'-dichorobenzidine among painters using products containing the chemical, supporting that paint is a source of exposure.

COMMENT: There is another ATSDR document on this facility not included in the references SELF-TESTING FOR HEMATURIA AMONG A POPULATION OF COMMUNITY RESIDENTS, WORKERS, AND FAMILY MEMBERS WITH POTENTIAL EXPOSURE TO BLADDER CARCINOGENS AT THREE LOCATIONS

IN MICHIGAN December 1999 publication number PB99-159840

RESPONSE: *This comment refers to the last paragraph in section 5.6 (General Population Exposure) regarding the ATSDR 1996 source. The study the peer reviewer refers to was reviewed and the information pertaining to 3,3'-dichlorobenzidine is from the 1996 ATSDR assessment of the Bofors-Nobel factory in Michigan. This study was not added as a reference, as it was confirmed to contain the same information as the ATSDR (1996) study already cited in the profile.*

COMMENT: Don't understand the use of the word versus here

RESPONSE: *This comment refers to the subtitles of figure 6-1 in section 6.1 (Existing Information on Health Effects). The text in the figure says "The majority of the studies examined oral exposure in animals (versus humans). Following review of the comments on the boilerplate text, ATSDR did not incorporate the edits. The boilerplate comments are collected for later review, because boilerplate changes are made to all profiles after a collective review.*

COMMENT: As stated in Section 2.4, this study does not address respiratory effects

RESPONSE: *This comment refers to the Acute-Duration MRLs paragraph in section 6.2 (Identification of Data Needs). The sentence the peer reviewer refers to states "One study in humans showed that the compound may cause respiratory effects when inhaled and that application of 3,3'-dichlorobenzidine base causes skin irritation (Gerarde and Gerarde 1974)." ATSDR agrees with the reviewer's edits and the following edits were made to the Acute-Duration MRLs paragraph in Section 6.2:*

> *"One study in humans showed that application of 3,3'-dichlorobenzidine base causes skin irritation (Gerarde and Gerarde 1974). The limited information in humans is insufficient to conclusively identify target organs, other than the skin, following exposure by any route. Acute-duration exposure can cause eye damage (erythema, pus, cornea1 opacity) in rabbits following conjunctival application. However, the relevance of these findings for the general population is unknown, since conjunctival application is not a likely route of exposure, and inhalation exposure is unlikely. 3,3'-Dichlorobenzidine can be lethal following oral and dermal exposure at very high doses. In most animal studies, comprehensive gross and histopathological evaluations have not been conducted, and clinical signs have not been monitored. Such studies may provide insight into systemic toxicity and potential health threats associated with acute-duration exposure. With the exception of effects caused by direct contact of 3,3'-dichlorobenzidine with the skin or the eyes, the limited pharmacokinetic data do not suggest route-specific target organs. The available data were inadequate for derivation of either inhalation or oral acute MRLs."*

COMMENT: At least two of the mortality studies included non-cancer endpoints

RESPONSE: *This comment refers to the Chronic-Duration MRLs paragraph in section 6.2 (Identification of Data Needs). The sentence the peer reviewer refers to states "No studies were located that examined no-cancer end points in humans following chronic exposure to 3,3'-Dichlorobenzidine." ATSDR agrees with the reviewer's edits and the following edits were made to the Chronic-Duration MRLs paragraph in Section 6.2:*

> *"Studies examining noncancer end points in humans following chronic exposure to 3,3'-dichlorobenzidine are limited and insufficient for derivation of a chronic MRL. Available chronic-duration oral studies provide information regarding systemic and carcinogenic effects in rats and dogs (Stula et al. 1975, 1978). These studies employed one dose level and the toxicological parameters measured were limited. Serious effects were seen at the lowest dose tested. The inadequacies of these studies precluded derivation of a chronic oral MRL. No chronic-duration animal inhalation or dermal exposure studies were located. Well conducted chronic-duration inhalation, dermal, and oral studies involving low-dose exposure in animals might provide dose-response data on potential systemic effects that could be extrapolated to humans. The available data are insufficient to establish a relationship between the concentration of 3,3'-dichlorobenzidine and/or its metabolites in the body and the levels that are associated with adverse effects. Studies that provide data on the body burden of 3,3'-dichlorobenzidine associated with toxicity may prove useful.*
>
> *Various studies have assessed the potential carcinogenicity of 3,3'-dichlorobenzidine in workers exposed to it (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). However, many confounders have rendered the results inconclusive. A major difficulty in such studies is the simultaneous exposure to several potential or known carcinogens. The carcinogenicity of 3,3'-dichlorobenzidine has been well established in animals after oral administration of the compound (Osanai 1976; Pliss 1959, 1963; Stula et al. 1975, 1978), but no information is available regarding inhalation and dermal exposure. There is suggestive evidence that 3,3'-dichlorobenzidine may cause cancer in animals when applied dermally since tumors were found in rats injected with the compound subcutaneously (Pliss 1963). Of particular interest would be additional studies, using relevant routes of exposure, to confirm the findings that 3,3'-dichlorobenzidine causes cancer in offspring of rats injected with the chemical subcutaneously during pregnancy (Golub et al. 1975)"*

COMMENT: As stated previously this study did <u>not</u> examine these end points

RESPONSE: *This comment refers to the Health Effects paragraph in section 6.2 (Identification of Data Needs). The paragraph originally states, "One human study examining neurological, gastrointestinal, and respiratory effects among occupationally exposed populations, was unable to distinguish if the effects observed were due to 3,3'-dichlorobenzidine (and its salts) exposure, or some other chemical in the workplace (Gerarde and Gerarde 1974)." As previously stated, human health effects reported from the Gerarde and Gerarde (1974) study were edited throughout the text. In response to this specific comment, the following edits were made in Section 6.2:*

"Few studies on human exposure to 3,3'-dichlorobenzidine were located, especially regarding noncancerous endpoints for all exposure routes. There is a need for studies examining noncancerous endpoints for 3,3'-dichlorobenzidine, particularly inhalation and dermal routes, which are more likely for workers. There is also need of human studies that identify the pathways of 3,3'-dichlorobenzidine in the human body (see Absorption, Distribution, Metabolism, and Excretion below) to better distinguish if observed effects are due to the chemical. Additionally, no animal or human studies identified a specific target organ for 3,3'-dichlorobenzidine; studies examining this are needed."

COMMENT: Adducts are a measure of exposure rather than a toxicological effect on hemoglobin

RESPONSE: *This comment refers to the statement in the hematological health effects paragraph in section 6.2 (Identification of Data Needs) that "Laboratory studies on animals strongly suggest a relationship between oral exposure to 3,3'-dichlorobenzidine and the formation of hemoglobin adducts (Birner et al. 1990; Joppich-Kuhn et al. 1997; Lee and Shin 2002)." ATSDR agrees with the reviewer and after further review adducts were taken out of sections describing it as a health effect resulting from 3,3'-dichlorobenzidine exposure. A discussion of adducts as a biomarker of exposure is included in Section 3.3.1 of the profile.*

COMMENT: Same comment as before about this study. They reported typical reasons workers came to clinic, which is true across all industries

RESPONSE: *This comment refers to the statement in the neurological health effects paragraph in section 6.2 (Identification of Data Needs) that says, "Workers exposed to 3,3'-dichlorobenzidine (and other chemicals) complained of headache and dizziness (Gerarde and Gerarde 1974)." Statements cited from the Gerarde and Gerarde (1974) study were edited and deleted throughout upon further review of the study. In response to this specific comment, the following edits were made to the Neurological paragraph in Section 6.2:*

"The nervous system has not been evaluated after exposure to 3,3'-dichlorobenzidine. A chronic-duration oral study in dogs reported convulsions in one of six dogs treated orally with 3,3'-dichlorobenzidine (Stula et al. 1978). Upon necropsy, the authors noticed slight neuronal degeneration in tissues (unspecified) of the nervous system in the convulsing dog. However, this effect was seen in only one of the six dogs and only one dose level was tested. The limited information available does not suggest that 3,3'-dichlorobenzidine is a neurotoxicant. However, any future long-term toxicity study on 3,3'-dichlorobenzidine in animals should include histological evaluation of representative elements of the nervous system. Furthermore, evaluation of neurological end points in offspring from animals exposed during gestation would provide information that may be relevant to children of pregnant women exposed to 3,3'-dichlorobenzidine in the workplace."

COMMENT: These uses not include in the use section 5.2.3.

RESPONSE: *The comment is in response to the following statement in the Epidemiology and Human Dosimetry Studies paragraph in section 6.2 (Identification of Data Needs): "The potential for*

occupational exposure exists in the use of 3,3'-dichlorobenzidine in the synthesis of 3,3'-dichlorobenzidine-based pigments for printing ink applications and to a lesser extent in paints." The stated uses here are the "3,3'-dichlorobenzidine-based pigments," and this use is stated in Section 5.2.3. No further edits have been made in response to this comment.

***C*OMMENT:** Rewrote the sentence to since opposite conclusion is true base on the limitations of epidemiology in examining risk of low level exposure

RESPONSE: *This comment refers to the sentence in the Epidemiology and Human Dosimetry Studies paragraph in section 6.2 (Identification of Data Needs) that says "Epidemiological studies of people who live in areas where 3,3'-dichlorobenzidine has been detected in groundwater, near industries releasing 3,3'-dichlorobenzidine, or near hazardous waste sites could provide information on whether 3,3'-dichlorobenzidine exposure produces effects in humans." ATSDR agrees and the following edits were made in the Epidemiology and Human Dosimetry Studies section in Section 6.2:*

> *"The potential for occupational exposure exists in the use of 3,3'-dichlorobenzidine in the synthesis of 3,3'-dichlorobenzidine-based pigments for printing ink applications and to a lesser extent in paints. Dermatitis is the only non-cancerous adverse health effects that appears to be associated with 3,3'-dichlorobenzidine exposure which is attributed to a manufacturing process change that resulted in exposure to 3,3'-dichlorobenzidine-freebase (Gerarde and Gerarde 1974). Studies of occupationally exposed individuals have been complicated by the fact that there is usually simultaneous exposure to other chemicals. Based on available data, the potential for nonindustrial exposure to the general population by air, soil, or water is expected to be negligible. Epidemiological studies of people who live in areas where 3,3'-dichlorobenzidine has been detected in groundwater, near industries releasing 3,3'-dichlorobenzidine, or near hazardous waste sites are not likely to provide information on whether 3,3'-dichlorobenzidine exposure produces effects in humans, given the likelihood that the levels of exposure to these populations has been low. In the unlikely event that exposure of the general population (in the past or present) to 3,3'-dichlorobenzidine occurs, individuals should be monitored for dermal effects (as reported earlier by Gerarde and Gerarde 1974).*
>
> *No studies were located that monitored human tissues for content of 3,3'-dichlorobenzidine or its metabolites. 3,3'-Dichlorobenzidine is excreted in urine. If 3,3'-dichlorobenzidine and metabolites can be detected and correlated with exposure, it may be possible to correlate urinary levels of 3,3'-dichlorobenzidine or its metabolites, with systemic effects."*

COMMENT: Deleted given over reading of Gerard article

RESPONSE: *This comment refers to the following sentence in the Epidemiology and Human Dosimetry Studies paragraph in section 6.2 (Identification of Data Needs): "In the unlikely event that exposure of the general population (in the past or present) primarily to 3,3'-dichlorobenzidine is identified, individuals should be monitored for gastrointestinal, respiratory, dermal, and neurological effects (as reported earlier by Gerarde and Gerarde 1974)." The peer reviewer suggested rewriting the sentence to say "In the unlikely event that exposure of the general population (in the past or present) primarily to 3,3'-dichlorobenzidine is identified, individuals should be monitored for dermal effects (as reported earlier by Gerarde and Gerarde 1974)." As previously stated, ATSDR agrees with the reviewer's*

statements regarding the Gerarde and Gerarde (1974) study. In response to this specific comment, the following edit was made:

> *"In the unlikely event that exposure of the general population (in the past or present) to 3,3'-dichlorobenzidine occurs, individuals should be monitored for dermal effects (as reported earlier by Gerarde and Gerarde 1974)."*

COMMENT: No data in this report to support this statement

RESPONSE: *This comment refers to the discussion of releases in the Production, Import/Export, Use, Release, and Disposal paragraphs in section 6.2 (Identification of Data Needs). The paragraph states "3,3'-Dichlorobenzidine is most likely to be found in sediments and soils near current or former industrial sites where the chemical was used. Since the chemical's use in U.S. manufacturing has decreased, releases to the environment are expected to be very low." Levels of 3,3'-dichlorobenzidine were primarily measured in soils near NPL sites as shown in Table 5-4, citing 2019 SPL data. No other recent data has been located on 3,3'-dichlorobenzidine levels in soils and sediments, besides one study reporting data from as late as 2003. As stated in the profile, 3,3'-dichlorobenzidine is no longer used in the United States and therefore it is assumed that releases to soil and sediment are expected to be much lower now. The text in section 6.2 was kept as is.*

COMMENT: First time mentioned as a source of exposure

RESPONSE: *The reviewer is referring to the statement regarding regulatory limitations for use in electric steam power generation in Chapter 7 (Regulations and Guidelines). This use has been added to Section 5.2.3 as follows:*

> *"3,3'-Dichlorobenzidine has been detected in the wastewater of metal finishing operation facilities and oilfield operations (See Section 5.5), suggesting they are used in these industries, but no specific information on its use is available. Additionally, the chemical is likely used in steam electric power generation based on its EPA regulatory limitation (EPA 2018a)."*

COMMENT: Should say:

No PEL established "Exposure to be controlled through the required use of engineering controls, work practices and personal protective equipment.."

RESPONSE: *This comment refers to the OSHA PEL entry in table 7-1 in Chapter 7 (Regulations and Guidelines). ATSDR agrees and the reviewer's edit was added to Table 7-1 as follows:*

OSHA	PEL (8-hour TWA) for general industry, shipyards and construction, and construction	No PEL established. Exposure should be controlled through the required use of engineering controls, work practices, and personal protective equipment.	OSHA 2016

COMMENT: Should say:

No REL established " recommends that exposures to carcinogens be limited to the lowest feasible concentration."

RESPONSE: *This comment refers to the NIOSH REL entry in table 7-1 in Chapter 7 (Regulations and Guidelines). ATSDR agrees and the reviewer's edit was added to Table 7-1 as follows:*

NIOSH	REL (up to 10-hour TWA)	No REL established. Exposures to carcinogens should be limited to the lowest feasible concentration.	NIOSH 2016

COMMENT: Edited some of the epidemiological definitions and added one.

RESPONSE: *This comment refers to Appendix E (Glossary.) The peer reviewer edited the definitions for cohort study, odds ratio (OR), standardized mortality ratio (SMR) and added definitions for standardized incidence ratio (SIR) and retrospective-prospective study. Following review of the comments on the boilerplate text, ATSDR did not incorporate the edits. The boilerplate comments are collected for later review, because boilerplate changes are made to all profiles after a collective review.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

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

COMMENT: YES, in that the main concern lies with industrial workers exposed to 3,3'-dichlorobenzidine (DCB) following its manufacture and its use in dye/pigment production. Oral, dermal, and inhalation exposures are most likely.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Bearing in mind that human studies are deemed inadequate to stand alone as the basis for establishing whether DCB poses a health risk to humans, results obtained in multiple animal species provide the basis for a "possible human carcinogen" designation.

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES – as doses, routes, types are given

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

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

COMMENT:

- Conclusions reflect little support for cancer, neurological, and hematological effects in humans.
- DCB is mutagenic, a carcinogen in animal species, but is not a clear carcinogen in humans.

RESPONSE: *No revisions were suggested.*

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

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

COMMENT: YES, both are evident.

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, both are evident.

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, this was evident.

RESPONSE: *No revisions were suggested.*

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

COMMENT: NO

RESPONSE: *No revisions were suggested.*

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

COMMENT: NO

RESPONSE: *No revisions were suggested.*

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

COMMENT: Details are included in lines 345-356 and Appendix C

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, agree

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, where known

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, appropriate

RESPONSE: *No revisions were suggested.*

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

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

COMMENT: Generally, YES

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, to the reviewer’s knowledge

RESPONSE: *No revisions were suggested.*

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

COMMENT: Generally YES; but lines 889-897 contain a confusing mixture of human and animal studies

RESPONSE: *This comment refers to the following text in section 3.2 (Children and Other Populations that are Unusually Susceptible): "There is no information regarding pharmacokinetics of 3,3'-dichlorobenzidine in children nor it is known whether 3,3'-dichlorobenzidine can be stored and excreted in breast milk. Although there have been no direct measurements to determine whether 3,3'-dichlorobenzidine can cross the placenta, two studies provide some indirect evidence that it or its metabolites do. In one study, 3,3'-dichlorobenzidine was orally administered to pregnant mice, which resulted in the induction of micronuclei in the liver of fetuses (Cihak and Vontorvoka 1987). In another study, pregnant mice were subcutaneously administered 3,3'-dichlorobenzidine, resulting in abnormal growth of the kidneys explanted from the fetuses (Shabad et al. 1972). No information was located on whether 3,3'-dichlorobenzidine can be stored in maternal tissues and be mobilized during pregnancy or lactation, or whether it can reach parental germ cells." To clarify the discussion of human and animals studies, the text was edited as follows:*

> *"There is no information regarding pharmacokinetics of 3,3'-dichlorobenzidine in children nor it is known whether 3,3'-dichlorobenzidine can be stored and excreted in breast milk. There have been no direct measurements in either humans or animals to determine whether 3,3'-dichlorobenzidine can cross the placenta. Two animal studies provide some indirect evidence that 3,3'-dichlorobenzidine or its metabolites do."*

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

COMMENT: First, it would be helpful to clarify how children are/would be exposed to DCB.

RESPONSE: *Possible sources of exposure for children to 3,3'-dichlorobenzidine are discussed under Section 5.6 General Population Exposure. There was no information on specific exposures to children, however general assumptions were stated such as exposure to paint chip debris. Child health and developmental effects are discussed in Section 3.2 Children and Other Populations that are Unusually Susceptible. No further edits were made to the text in response to this comment.*

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

COMMENT: Unclear what "other populations" are involved

RESPONSE: *Populations discussed throughout the profile were primarily workers in occupational settings working with the chemical and communities near hazardous waste and manufacturing facilities producing or using the chemical. These groups are discussed throughout the profile and specifically*

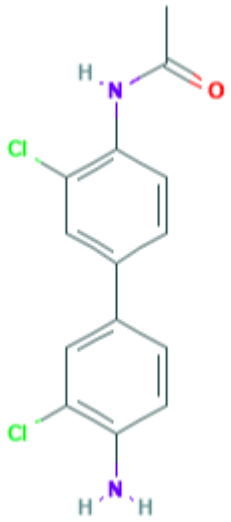
noted in Section 5.7 Populations with Potentially High Exposures. No further edits were made to the text in response to this comment.

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

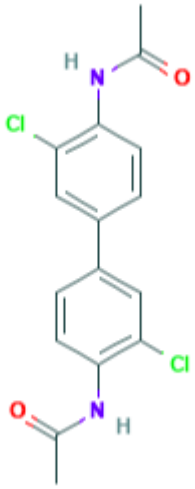
COMMENT: YES, in that specific names are given. It would be helpful, however, to indicate the corresponding molecular structures (formulas), expecially since this Tox Profile uses both N,N- and N,N'- designations which carry different meanings.

RESPONSE: *The nomenclature of N,N'-diacetyl-3,3'-dichlorobenzidine was corrected for consistency throughout the profile. The chemical structures of the 3,3'-dichlorobenzidine metabolites, N-acetyl-3,3'-dichlorobenzidine and N,N'-diacetyl-3,3'-dichlorobenzidine, were added in Chapter 3, Figure 3-1 as follows:*

N-acetyl-3,3'-dichlorobenzidine



N,N'-diacetyl-3,3'-dichlorobenzidine



The structures of the potential nitroso intermediate and the hydroxy- intermediates were also added as Figure 3-2 and Figure 3-3, as follows:

Figure Error! No text of specified style in document.**-1. Potential Form of the Nitroso Intermediate**

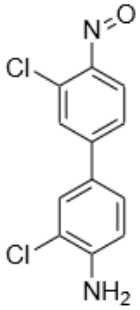
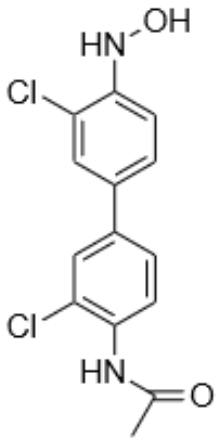
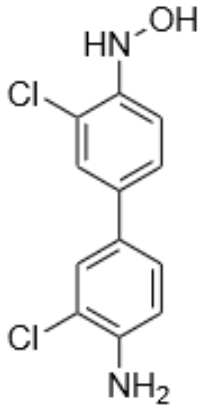


Figure Error! No text of specified style in document.**-2. Hydroxy- Metabolites of 3,3'-Dichlorobenzidine**

N-hydroxy-N'-acetyl-dichlorobenzidine



N-hydroxy-Dichlorobenzidine



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

COMMENT: Yes, in that they require the substance in order to be produced.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Main point seems to be that an interaction with BHT enhances mutagenicity and DNA binding

RESPONSE: *No revisions were suggested.*

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

COMMENT: Details pertaining to synergistic interactions with BBN are discussed.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

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

COMMENT:

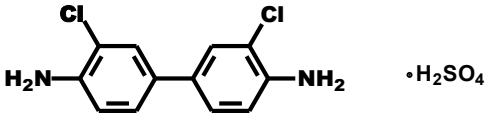
- In Table 4.1, molecular information pertaining to DCB is correct.
- In Table 4.2, water solubility data is one of multiple values covered in this Profile. It reflects sparingly low water solubility, which is more reasonable than the suggestion of water insolubility mentioned in section 5.3.2.

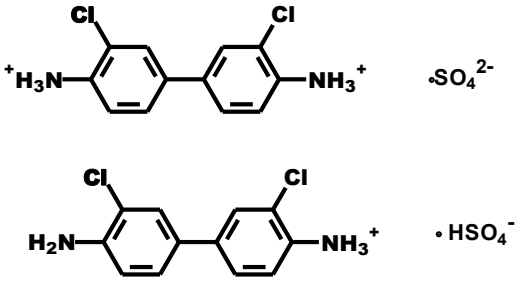
RESPONSE: *This inconsistency was resolved in the profile based on the comments addressed below under Annotated Comments. ATSDR agrees that it is more accurate and reasonable to state that 3,3'-dichlorobenzidine has low water solubility.*

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

COMMENT:

- Molecular structure given for DCB sulfate should reflect the fact that sulfuric acid is a dibasic acid, requiring only one mole of acid per mole of DCB. Thus, the Table 4.1 structure is better designated as one of the following three options:





- Note that the thirst option would indicate that only one-half mole of sulfuric acid was used per mole of DCB, giving the bisulfate rather than sulfate salt.
- This also means that the molecular mass should be changed to 351.20 (Table 4.2) for $C_{12}H_{12}Cl_2N_2O_4S$.

RESPONSE: *Following review of the reviewer's recommendation, it was determined that there were 2 distinct forms listed under the original entry for "3,3'-dichlorobenzidine sulfate." Table 4-1 and Table 4.2 were edited to list 3,3'-dichlorobenzidine dihydrogen bis(sulphate) and 3,3'-dichlorobenzidine sulphate separately in distinct columns as they have distinct CASRNs, chemical structures, and molecular weights.*

Chapter 5. Potential for Human Exposure

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

COMMENT: In line 1048, it would be good to replace "which" with "and the resulting hydrazobenzene derivative"

RESPONSE: *This comment refers to the statement in section 5.2.1 (Production) "3,3'-Dichlorobenzidine is commercially produced by reduction of o-nitrochlorobenzene with zinc dust and sodium hydroxide solution, which is rearranged with dilute hydrochloric acid or sulfuric acid to form 3,3'-dichlorobenzidine (Schwenecke and Mayer 2000; DCMA 1989; Sax 1987)." ATSDR agrees with the reviewer's edit and the following edit was made in Section 5.2.1 Production:*

> *"3,3'-Dichlorobenzidine is a chlorinated primary aromatic amine (NTP 2016; WHO 1998). The primary anthropogenic source of 3,3'-dichlorobenzidine is pigments and dyes. 3,3'-Dichlorobenzidine is no longer used to manufacture dyes in the United States (CPMA 1998). 3,3'-Dichlorobenzidine is commercially produced by reduction of o-nitrochlorobenzene with zinc dust and sodium hydroxide solution, and the resulting hydrazobenzene derivative is rearranged with dilute hydrochloric acid or sulfuric acid to form 3,3'-dichlorobenzidine (Schwenecke and Mayer 2000; DCMA 1989; Sax 1987). Commercial supplies are usually provided in the form of the dihydrochloride salt because of its greater stability."*

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

COMMENT: Seems reasonable

RESPONSE: *No revisions were suggested.*

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

COMMENT: Points made are clear and logical, except for the suggestion of covalent bond formation with humic and fulvic components in soils. Ionic bond formation seems more logical, especially at room temperature.

RESPONSE: *The suggestion of covalent bond formation with humic and fulvic compounds in soils is supported by the Boyd et al. (1984) study. There are no sources located to support changing the statement to state ionic bond formation. No further edits were made in response to this comment.*

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

COMMENT:

- Information given is reasonable, except for the 90 sec half-life for the conversion of DCB to MCB using sunlight.
- There are differences in previously used units that require revision for consistency, such as cu-m instaed of m^3.
- Units in line 1398 requires attention. The unit 654 L seems odd.

RESPONSE: *3,3'-Dichlorobenzidine has been described as photolabile in water. The 90-second half-life reported in the Banerjee et al. (1978) was determined for 3,3'-dichlorobenzidine in water exposed to natural sunlight, as opposed to artificial light which is described in the preceding statement. No further edits were made in response to the reviewer's first bullet point.*

The EPA-estimated hydroxyl radical reaction rate constant is reported as cu cm/molecule-sec at 25 degrees Celsius. The units were kept consistent with how reported. No further edits were made in response to the reviewer's second bullet point.

The reviewer is noting the statement: "Water concentrations near the Toronto pigment site were from 2.60 µg/L up to 654 µg/L." The unit is reported as "ug/L" and all units in this paragraph are consistent. No further edits were made in response to the reviewer's third bullet point.

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

COMMENT: Meaning of "sediment pore water" in line 1405 is unclear.

RESPONSE: *This comment refers to the statement in section 5.5.2 (Water) that says "Harden et al. (2005) detected up to 26.8 µg/L of 3,3'-dichlorobenzidine in the water phase and up to 8.71 µg/L in sediment pore water." Harden et al. (2005) states that 3,3'-dichlorobenzidine was measured in "sediment pore water." This refers to water that is found within sediment. No further edits were made in response to this comment.*

Chapter 6. Adequacy of the Database

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

COMMENT: NO

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES, especially data needed to establish whether children and adults are equally succeptible to DCB toxicity and whether sub-cu and dermal application of DCB give equivalent results.

RESPONSE: *No revisions were suggested.*

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

COMMENT: YES

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: NO

RESPONSE: *No revisions were suggested.*

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

COMMENT: NO

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

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

COMMENT: YES, in that results from human studies are inadequate. This accounts for the current EPA and IARC clasifications. It is evident that DCB is significantly different from the parent compound benzidine.

RESPONSE: *No revisions were suggested.*

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

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

COMMENT: This reviewer is not aware of derived MRLs.

RESPONSE: *No revisions were suggested.*

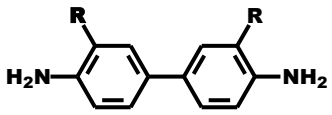
QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: None.

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

COMMENT: Reviewer 2: It would be good to include the molecular formula for DCB and to discuss this compound in the context of benzidine and other homologs designated as human carcinogens.



R = H (Benzidine)
R = CH_3 (3,3'-Dimethylbenzidine)
R = OCH_3 (3,3'-Dimethoxybenzidine)
R = Cl (3,3'-Dichlorobenzidine)

RESPONSE: *The peer reviewer suggests this addition in section 1.1 (Overview and U.S. Exposures). The molecular formula and chemical structure of 3,3'-DCB and its salts are presented in Table 4-1, Chapter 4. Additional detail in section 1.1 (Overview and U.S. Exposures) was added to state that benzidine is structurally similar to benzidine and other homologs:*

> *"3,3'-Dichlorobenzidine is a synthetic chlorinated primary aromatic amine structurally similar to benzidine and other homologs designated as human carcinogens. It is used in the production of azo dyes and pigments in the textile, inks, plastics, rubber, and leather industries."*

The profile does not discuss a comparison of the toxicity between 3,3'-dichlorobenzidine and other structurally similar compounds as this is not within the scope of the Profile. When relevant, benzidine is discussed in relation to 3,3'-dichlorobenzidine, such as the degradation of the chemical to benzidine in anaerobic mixtures of sediment/water and in the metabolism and metabolite formation discussion in Chapter 3, which includes the following:

> *"In the case of benzidine, a structurally similar compound, Zwirner-Baier and Neumann (1998) statea, "Martin et al. (1982) identified the DNA-adduct as N-(deoxyguanosin-8-yl)-N'-acetyl-benzidine and proposed the N-hydroxy derivative of the N'-monoacetamide as the proximate genotoxin" (Zwirner-Baier and Neumann 1998; p. 499). It is not definitively known where on DNA 3,3'-dichlorobenzidine derivatives bind to form adducts. Iba (1989) explored the question of where on DNA 3,3'-dichlorobenzidine metabolites bind and found that 3,3'-dichlorobenzidine is metabolized by rat liver microsomes to derivatives that bind covalently to added deoxyguanosine at a neutral pH, but also point out that this finding is not consistent with other carcinogenic arylamines. This indicates the mechanism of binding may be different than with benzidine."*

COMMENT: This explanation would have been helpful to understanding Table 1-1 of chapter 1.

RESPONSE: *The peer reviewer refers to the three exposure periods discussed in section 2.1 (Introduction):" acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days)." A statement*

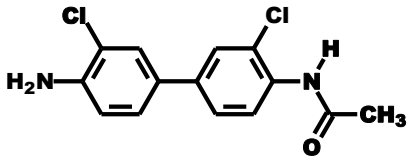
was added to Chapter 1 in section 1.2 (Summary of Health Effects) explaining exposure durations as follows:

> *"Exposure durations are defined as follows: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days)."*

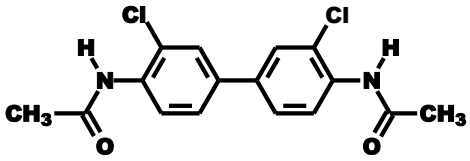
COMMENT: 6.5?

RESPONSE: *The reviewer is referring to the following statement in section 2.4 (Respiratory): "Dyspnea was observed in 1 of 6 female dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 6.6 years, which likely resulted as a secondary effect of liver disease that this dog was experiencing." It was confirmed in Stula et al. (1978), that 6.6 years is correct.*

COMMENT: It will be helpful to insert structures for the acetylated DCB derivatives



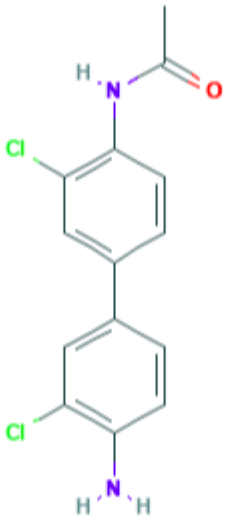
N-Acetyl-3,3'-dichlorobenzidine

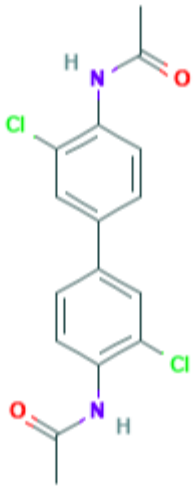


N,N'-Diacetyl-3,3'-dichlorobenzidine

RESPONSE: *The peer reviewer suggests the additions of the above figures to section 2.7 (Hematological). The peer reviewer suggested inserting these figures and left the comment on the figures. The metabolites provided by the reviewer were added into Chapter 3, Figure 3-1 as follows:*

N-acetyl-3,3'-dichlorobenzidine N,N'-diacetyl-3,3'-dichlorobenzidine





COMMENT: Why not specify?

RESPONSE: *This comment refers to the strains discussed in section 2.20 (Genotoxicity). The original statement reads "One study (Lazear et al. 1979) reported negative results for gene mutation both with and without activation in one strain of Salmonella typhimurium and positive results in another strain with and without activation." The specific strain being referred to was added. The following statement in Section 2.20 Genotoxicity was edited:*

> *"One study (Lazear et al. 1979) reported negative results for gene mutation both with and without activation in the TA100 strain of Salmonella typhimurium and positive results in the TA98 strain with and without activation."*

COMMENT: Why not specify?

RESPONSE: *This comment refers to the strains discussed in section 2.20 (Genotoxicity). The original statement reads "One study (Lazear et al. 1979) reported negative results for gene mutation both with and without activation in one strain of Salmonella typhimurium and positive results in another strain with and without activation." The specific strain being referred to was added. The following statement in Section 2.20 Genotoxicity was edited:*

> *"One study (Lazear et al. 1979) reported negative results for gene mutation both with and without activation in the TA100 strain of Salmonella typhimurium and positive results in the TA98 strain with and without activation."*

COMMENT: Is there a difference between "bladder" and "urinary bladder"?

RESPONSE: *This comment refers to section 2.20 (Genotoxicity). The original text in question states "Among cells of eight organs examined (stomach, colon, liver, kidney, bladder, lung, brain, and bone*

marrow), 3,3'-dichlorobenzidine induced a statistically significant increase in DNA damage in the stomach, liver, urinary bladder, lung, brain, and bone marrow compared with controls." Bladder and urinary bladder had been used interchangeably throughout the profiles. All instances of "urinary bladder" in the text were changed to "bladder" for consistency.

COMMENT: Dermal?

RESPONSE: *This comment refers to all of section 3.1.1 (Absorption). Section 3.1 discusses absorption through all exposure routes, including dermal. No edits were made in response to the reviewer's comment.*

COMMENT: Possible from dermal absorption?

RESPONSE: *This comment refers to the second paragraph in section 3.1.1 (Absorption) and the sentence that states "3,3'-Dichlorobenzidine has been detected in the urine of workers in 3,3'-dichlorobenzidine -handling plants under conditions which favored inhalation of 3,3'-dichlorobenzidine-bound particulate matter (Handke et al. 1986; London and Boiano 1986; Meigs et al. 1954)." Dermal absorption is discussed in the last paragraph of Section 3.1.1. No edits were made in response to the reviewer's comment.*

COMMENT: This point would have been helpful for introducing Chapter 1!

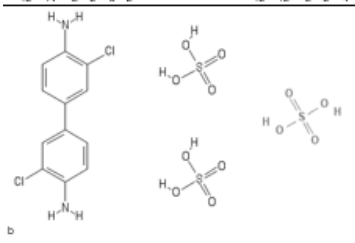
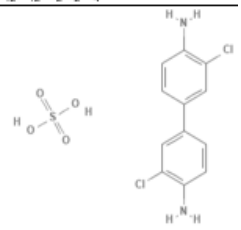
RESPONSE: *This comment refers to the statement in 3.1.6 (Animal-to-Human Extrapolations) "…however, there is concern about occupationally exposed subjects because of 3,3'-dichlorobenzidine's structural similarity with the known human and animal carcinogen benzidine." ATSDR agrees with the reviewer's comment, and a statement regarding 3,3'-dichlorobenzidine's structural similarity to benzidine was added to Section 1.1 as follows:*

> *"3,3'-Dichlorobenzidine is a synthetic chlorinated primary aromatic amine structurally similar to benzidine, used in the production of azo dyes and pigments in the textile, inks, plastics, rubber, and leather industries."*

COMMENT: This is the formula for suggested structure:

$C_{12}H_{12}Cl_2N_2O_4S^b$

RESPONSE: *This comment refers to Table 4-1 and the chemical formula for 3,3'-dichlorobenzidine sulfate, originally written in the profile as $C_{12}H_{14}Cl_2N_2O_8S_2$. As previously noted, two distinct columns were made in Table 4-1 for 3,3'-dichlorobenzidine dihydrogen bis(sulphate) and 3,3'-dichlorobenzidine sulphate, as follows:*

3,3'-Dichlorobenzidine dihydrogen bis(sulphate)	3,3'-Dichlorobenzidine sulphate
(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-, sulfate (1:2)[a]	3,3'-Dichlorobenzidine sulphate; Benzidine, 3,3'-dichloro-, sulfate; (1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-, sulfate (1:1)[a]
$C_{12}H_{14}Cl_2N_2O_8S_2$[a]	$C_{12}H_{12}Cl_2N_2O_4S$[a]
	
64969-34-2	64414-68-2 74332-73-3

COMMENT: See suggested structures below.

RESPONSE: *This comment refers to Table 4-1 and the chemical structures for 3,3'-dichlorobenzidine dihydrochloride and 3,3'-dichlorobenzidine sulfate. See previous response for edits made to Table 4-1.*

COMMENT: This should be Table 4-3.

RESPONSE: *The reviewer is referring to the second page of Table 4-2. Table 4-2 extends over two pages. No edits were made in response to the reviewer's comment.*

COMMENT: See Table 4.2 and line 1202

RESPONSE: *This comment refers to the statement in the second paragraph of section 5.3.2 (Water) that says "The free base from of 3,3'-dichlorobenzidine is insoluble in water, and will rapidly absorb and bind to sediment and particulate matter (NTP 2011)." The reader points to the line in the water paragraph of section 5.4.1 (Transport and Partitioning) that says 3,3'-dichlorobenzidine has low solubility in water. This statement in section 5.3.2 was corrected to state "slightly soluble in water."*

Comments provided by Peer Reviewer #3

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

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

COMMENT: The only adverse health effect in humans, other than dermatitis, is apparently cancer. The possibility of bladder cancer in occupationally-exposed persons should be mentioned in Section 1.2.

RESPONSE: *Bladder cancer in occupationally exposed populations is discussed in Section 1.2 under Cancer. No further edits were made to the text. Please refer to the following:*

> ***"****The six human studies identified involved occupationally exposed populations. The findings of the studies are mixed, and they are not of high quality. Three of the six studies found no association between exposure and bladder cancer incidence (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975). However, these three studies had limited power to detect an exposure-related effect due to small sample size, short follow-up time, no control group, or co-exposure to other chemicals. Two of the six studies reported an increase in bladder tumors in workers exposed to either benzidine-based azo dyes (Myslak 1991) or arylamines (Ouellet-Hellstrom and Rench 1996). While the workers were exposed to 3,3'-dichlorobenzidine, they were exposed to other chemicals linked to bladder cancer. The sixth, Rosenman and Reilly (2004) identified one case of bladder cancer among workers exposed only to 3,3'-dichlorobenzidine in a chemical manufacturing plant. Among these same workers, an increased risk for lymphohematopoietic cancer was identified (standardized mortality ratio=6.62; 95% confidence interval 1.37-19.36). A limitation of this study is the lack of quantitative exposure data for the workers as only the number of years worked was provided. The evidence for carcinogenicity of 3,3'-dichlorobenzidine comes from studies on multiple animals and models. 3,3'-Dichlorobenzidine has been found to be carcinogenic in rats, mice, dogs, and equivocal in hamsters (Osanai 1976; Pliss 1959; Stula et al. 1975, 1978)."*

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

COMMENT: It is quite possible that sufficiently high, chronic exposure to DCB can cause bladder tumors in humans. Bladder cancer was reported in 2 epidemiology studies, as well as in rats and dogs. DCB appears to be metabolically activated in rodents and humans to moieties that produce hemoglobin and DNA adducts and manifestations of genotoxicity.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Descriptions of exposure conditions are adequate for this section of the document.

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

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

COMMENT: The health effect conclusions accurately/adequately reflect findings in the published scientific literature. It should be noted on page 27 of Section 2.2 that preponderance of results support the conclusion that DCB is genotoxic in most in vitro systems.

RESPONSE: *A summarizing phrase was added stating the 3,3'-dichlorobenzidine was genotoxic in most in vitro systems, as follows in Section 2.20: "As summarized in Table 2-3, 3,3'-dichlorobenzidine appeared genotoxic in most in vitro test systems employed."*

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

COMMENT: Deficiencies of existing epidemiology studies of DCB are detailed.

RESPONSE: *No revisions were suggested.*

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

COMMENT: The relatively few toxicology studies in animals were adequately described in the text of the Tox Profile. Comprehensive studies other than cancer bioassays were very limited in number.

RESPONSE: *No revisions were suggested.*

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

COMMENT: NA

RESPONSE: *No revisions were suggested.*

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

COMMENT: Data were inadequate in most instances to assess dose-response relationships.

RESPONSE: *No revisions were suggested.*

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

COMMENT: I was not able to locate published accounts of any additional toxicology studies that would be useful in evaluating toxicity or in deriving MRLs.

RESPONSE: *No revisions were suggested.*

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

COMMENT: I was not able to locate published accounts of any additional toxicology studies that would be useful in evaluating toxicity or in deriving MRLs.

RESPONSE: *No revisions were suggested.*

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

COMMENT: NA

RESPONSE: *No revisions were suggested.*

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

COMMENT: The few studies and sparse data were generally inadequate for identifying meaningful NOAELs and LOAELs.

RESPONSE: *No revisions were suggested.*

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

COMMENT: There needs to be a more comprehensive presentation of the experimental evidence (i.e., in vitro and in vivo genotoxicity, animal bioassay, and epidemiology) supporting and refuting the position that DCB is a human carcinogen. There also needs to be a more comprehensive presentation of findings

relevant to the most likely mechanism(s) of carcinogenicity. This latter subject might best be included in Section 3.1.3. See my Specific Comments. It would be helpful to compose a Figure to illustrate the cytochrome P450 oxidation and subsequent NADPH P450 reductase metabolic activation pathway (Wang and Guengerich, 2013; Iba, 1989) and the N-acetylation detoxification/elimination pathway.

RESPONSE: *The reviewer's specific comment was in section 3.1.3 (Metabolism as follows: "It would be helpful to include a figure showing the major metabolic pathways involving activation and inactivation. See Zwirner-Baier and Neumann (1998) and Wang and Guengerich (2013) for diagrams." The reviewer also provided the following citations:*

Iba, M.M. (1989). Activation of 3,3'-dichlorobenzidine: Enzymic basis and toxicological consequences. Drug Metab. Rev. 21(3):377-400.

Wang, K. and Guengerich, F.P. (2013). Reduction of aromatic and heterocyclic N-hydroxylamines by human cytochrome P4502S1. Chem. Res. Toxicol. 26(6):993-1004.

Structures of the metabolites were added to the section 3.1.3. discussion of adduct formation which was also expanded. The exact metabolites of DCB which bind to hemoglobin or DNA are not known. It is anticipated that it will behave similarly to benzidine, but there is enough evidence in the literature that this may in fact not be the case therefore a full metabolism figure was not added into the profile.. For example, Iba (1987) speculates that the activation of DCB may occur on the carbon atom instead or in addition to the nitrogen. The role of acetylation vs oxidation is also unclear, suggesting that both pathways may lead to reactive metabolite formation. A brief discussion of the similarities and differences with benzidine as briefly added to section 3.1.3 as follows:

> *"In the case of benzidine, a structurally similar compound, Zwirner-Baier and Neumann (1998) states, "Martin et al. (1982) identified the DNA-adduct as N-(deoxyguanosin-8-yl)-N'-acetyl-benzidine and proposed the N-hydroxy derivative of the N'-monoacetamide as the proximate genotoxin" (Zwirner-Baier and Neumann 1998; p. 499). It is not definitively known where on DNA 3,3'-dichlorobenzidine derivatives bind to form adducts. Iba (1989) explored the question of where on DNA 3,3'-dichlorobenzidine metabolites bind and found that 3,3'-dichlorobenzidine is metabolized by rat liver microsomes to derivatives that bind covalently to added deoxyguanosine at a neutral pH, but also point out that this finding is not consistent with other carcinogenic arylamines. This indicates the mechanism of binding may be different than with benzidine."*

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

COMMENT: The overall conclusion about the inadequacy of the toxicology database is valid. More could be said about the general mechanism of carcinogenesis (i.e., biotransformation of DCB to products (N-hydroxylamine and nitroso derivations of DCB) that alkylate DNA and hemoglobin) (Iba, 1989; Ghoshal and Iba, 1990).

RESPONSE: *The Ghoshal and Iba (1990) and Iba (1989) studies were reviewed and relevant information was added throughout Chapter 3 of the profile , including in Section 3.1.3 (Metabolism):*

> *"Joppich-Kuhn et al., (1997) found that the hemoglobin adducts formed with 3,3'-dichlorobenzidine are stable in vivo, and they persist for the life of the erythrocyte. In the case of DNA adducts, the lifespan of the DNA adducts varied by species and tissue type. Experiments in*

the liver, bladder epithelium and small intestinal epithelium of rats and mice following a single oral dose of 3,3'-dichlorobenzidine found that even one dose lead to extensive covalent DNA binding, and the rate of adduct removal did not vary between identified target and non-target tissues (Ghosal and Iba 1990). The authors speculate that peak binding may result in higher rates of carcinogenicity, however more research is needed to determine this. Specifically, Ghosal and Iba (1990) found that the half-life of the DNA adducts in the liver and in the bladder epithelium were similar in rats and mice (13-14 day) and about three times longer than the half-life in the intestinal epithelium.

Studies have further explored the enzymes involved in the metabolism of 3,3'-dichlorobenzidine. Evidence in rats and mice suggests 3,3'-dichlorobenzidine induces hepatic microsomal cytochrome P-450 (Iba 1989). Cytochrome P-450 inhibitors, α-naphthoflavone and SKF-525A, modified the hepatic microsomal metabolism of 3,3'-dichlorobenzidine. 3,3'-Dichlorobenzidine formed a complex with oxyferro-P-450 in microsomes, indicating metabolism occurred. N-acetyl-3,3'-dichlorobenzidine (acDCB) and azodichlorobenzidine (AzoDCB) were the primary formed metabolites, and are suggested useful indices of 3,3'-dichlorobenzidine metabolism by the study author (Iba 1989). NADPH enhances 3,3'-dichlorobenzidine covalent binding to polynucleosides in the presence of rat liver S9 in vitro. Cytochrome P-450 appears involved in the formation of reactive DCB species, as α-naphthoflavone inhibits this NADPH-dependent binding by 50% (Iba 1989).
Iba (1989) suggests the role of flavin-containing monooxygenase (FMO) in the microsomal metabolism of 3,3'-dichlorobenzidine as N-acetyl-3,3'-dichlorobenzidine is an extractable product with solvents. The role of FMO was examined as carbon monoxide, which does not inhibit FMO activity, and did not appear to effect microsomal metabolism of 3,3'-dichlorobenzidine. In the same study, the author found that peroxidases do not appear to play a role in metabolism of 3,3'-dichlorobenzidine, despite its roles in the metabolism of most carcinogenic arylamines.
Iba (1989) identified cytochrome P-450d, induced by 3,3'-dichlorobenzidine, and flavin-containing monooxygenase (FMO) as the primary enzymes responsible for forming mutagenic DCB derivates in microsomes. Rat liver microsomes were used as the source of activating enzymes and mutagenicity to TA98. Additional testing showed cytochrome P-450d formed both mutagenic and lipid-binding DCB derivatives. The same testing revealed FMO forms mutagenic, but not lipid-binding, 3,3'-dichlorobenzidine derivates. As similarly described in microsomal metabolism, carbon monoxide did not inhibit microsomal activation of 3,3'-dichlorobenzidine. Additionally, post-oxidative activation does not appear important to the hepatic activation of 3,3'-dichlorobenzidine attributed to its mutagenicity in TA98, which requires post-oxidative enzymes, and its activation by rat liver S9 preparation, which does not require these enzymes."

Text was also added in section 3.1.4 (Excretion):

"As noted in Section 3.1.3, N-acetylation appears to be a major metabolic path of 3,3'dichlorobenzidine in mammals. Iba (1989) noted the reduced lipophilicity of 3,3'-dichlorobenzidine effected by N-acetylation, likely decreasing access to active sites of microsomal oxidases and enhancing bioelimination of 3,3'-dichlorobenzidine (Iba 1989)."

The citation was also added in Section 6.2 (Identification of Data Needs):

"Genotoxicity. Available studies in animals and in bacterial systems show that 3,3'-dichlorobenzidine did alter genetic material (Ashby and Mohammed 1988; Bratcher and Sikka 1982; Chen et al. 2003, 2014; Chung et al. 2000; Cihak and Vontorkova 1987; Garner et al.

1975; Hering et al. 2018; Iba 1987; Imaoka et al. 1997; Lazear et al. 1979; Makena and Chung 2007; Sasaki et al. 1999; Savard and Josephy 1986; Shiraishi 1986; Wang et al. 2005).”

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

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

COMMENT: Discussion/description of the metabolic activation and inactivation of DCB should be expanded, in order to better understand the mechanism of carcinogenicity and factors that influence formation of adducts and genotoxicity. See my previous comments.

There is adequate description of the limited information available on absorption, distribution and excretion.

RESPONSE: *As stated in previous comments, ATSDR does not make recommendations on carcinogenicity but rather reports classifications by EPA, IARC and the U.S. Department of Health and Human Services. Additionally, the study Ghoshal and Iba (1990) recommended by the reviewer does not contain information to expand on discussion. The Iba (1989) study was reviewed and its information incorporated throughout Chapter 3 of the profile. Please see response to previous comment for the excerpt from the Profile containing the added information.*

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

COMMENT: NA

RESPONSE: *No revisions were suggested.*

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

COMMENT: There are not sufficient toxicokinetic data for a meaningful discussion of interspecies differences or similarities.

RESPONSE: *No revisions were suggested.*

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

COMMENT: I am not aware of any data on DCB relevant to children’s health.

RESPONSE: *No revisions were suggested.*

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

COMMENT: No information was found on susceptibility of pediatric or geriatric age-groups, other than the age-dependence of N–acetyltransferase and UDP-glucuronyl transferase activities.

RESPONSE: *No revisions were suggested.*

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

COMMENT: DCB's acetylated and nitroso metabolites apparently form adducts with cells in tissues, as well as with hemoglobin and with DNA of lymphocytes. These adducts are DCB-specific.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Adducts of exfoliated bladder cells can be considered biomarkers of effect, as adducts typically presage manifestation of genotoxicity.

RESPONSE: *The Lee et al. (2003) study, cited in the Profile, discusses the measurement of 3,3'-dichlorobenzidine metabolites extracted from DNA adducts extracted from exfoliated bladder epithelial cells as a biomonitoring tool for occupationally-exposed workers. There is insufficient evidence from this one study to make a determination that adducts of exfoliated bladder cells can be used as a biomarker of effect, as the role and correlation between the adduct formation and the mechanism of carcinogenicity is still being studied..*

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

COMMENT: The discussion of the limited information on interactions with other chemicals is adequate. It appears that DNA adductive and downstream genotoxic actions of DCB and nitrosamines may be additive or synergistic.

RESPONSE: *No revisions were suggested.*

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

COMMENT: The text does not address mechanisms of potential interactions.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

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

COMMENT: I am not aware of inaccuracies in the values or other information provided.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Information on DCB and its different salts is provided.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

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

COMMENT: Ample information on production, import, use and disposal is provided.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Details on the disposal of DCB and DCB-containing dyes appears to be limited to a single company (London and Boiano, 1986). Presentation of frequency of occurrence at NPL sites is clear, though the number of sites monitored for DCB is limited. How current are these data?

RESPONSE: *Disposal data were limited to the London and Boiano (1986) study. The NPL data presented in Figure 5-1 has been updated with the 2019 NPL data.*

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

COMMENT: The text appears to be comprehensive in its coverage of information on the transport, partitioning, transformation and degradation of DCB.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the

form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: Data are provided on DCB concentrations measured in water and soils in the proximity of manufacturing and release sites. DCB is apparently not detectable in the general environment. DCB, not its salts, is monitored. The quality of modeling data is mentioned.

RESPONSE: *No revisions were suggested.*

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

COMMENT: The text does a good job describing sources and pathways of exposure of occupationally-exposed individuals, as well as the general population. Workers and people living in the proximity of previous manufacturing/usage/disposal facilities obviously have the greatest potential for relatively high exposure.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

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

COMMENT: It is stated in lines 1568 and 1569 that pharmacokinetic data do not suggest route-specific target organs. DCM is known to be activated by cytochrome P450-mediated oxidation (Iba, 1989). Therefore, it would be anticipated that the liver would be a target organ for toxicity and carcinogenicity upon oral exposure.

Stula et al. (1978) described liver injury in a chronic dog study. An oral intermediate or chronic-duration study employing a range of doses would be useful to determine the dose- and time-dependency of hepatotoxicity in mice and/or rats.

RESPONSE: *The Iba (1989) was reviewed and further information was added in Section 3.1.3 of the profile regarding 3,3'-dichlorobenzidine induced cytochrome P-450 in rat liver microsomes.*

ATSDR agrees with the reviewer's recommendation of adding hepatic effects in Section 6.2 Identification of Data Needs. The following statement was added:

> *"**Hepatic.** Liver injury was seen in one of six dogs chronically exposed to 3,3'-dichlorobenzidine, noted by marked fatty change in the liver (Stula et al. 1978). All dogs exhibited modestly elevated GPT levels. Further intermediate or chronic oral animal studies testing a range of doses would help to determine the dose and time dependency of hepatotoxicity in animals."*

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

COMMENT: I concur with the statements in the text advocating a chronic oral toxicity experiment. I do not think that chronic inhalation or dermal studies are warranted, as these routes of exposure are unlikely to be important with environmental media.

The meaning of the complete sentence in lines 1609 & 1610 is unclear. Are the authors advocating human toxicokinetic studies for different exposure routes?

I agree with the stated needs and experimental approaches for neurological and developmental toxicity investigations, assuming DCB still has a high enough priority for funding.

Epidemiological studies of potential toxicological effects in the general population exposed to trace levels of DCM in drinking water do not seem to be worthwhile. Measurement of DNA adducts in lymphocytes or hemoglobin adducts might yield some useful/definitive data, should one or more of the adducts be slowly repaired (i.e., cumulative).

RESPONSE: *The reviewer is referring to the following sentence: "There is also a need of human studies that identify the pathways and of 3,3'-dichlorobenzidien in the human body, to better distinguish if observed effects are due to the chemical." The data need is stating a need for human toxicokinetic studies of different exposure routes. The sentence was changed to the following:*

> *"There is also need of human studies that identify the biological fate of 3,3'-dichlorobenzidine in the human body (see Absorption, Distribution, Metabolism, and Excretion below). to better distinguish if observed effects are due to the chemical. "*

The data needs for epidemiological studies states that exposure to populations near hazardous waste sites is low and further studies are likely not needed. No further edits were made in response to the reviewer's comments.

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

COMMENT: Data needs are presented in a neutral, non-biased or non-judgmental fashion.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

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

COMMENT: I am not aware of any additional guidelines or regulations that should be included.

RESPONSE: *No revisions were suggested.*

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

COMMENT: I did not find any that should be removed.

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

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

COMMENT: I agree that data are inadequate for derivation of MRLs. Risk assessments based on carcinogenesis would take precedence anyhow.

RESPONSE: *No revisions were suggested.*

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

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

COMMENT: NA

RESPONSE: *No revisions were suggested.*

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

COMMENT: NA

RESPONSE: *No revisions were suggested.*

Appendices

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

COMMENT: No comments

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

COMMENT: page 8, line 19: It should be pointed out that tumors have been found in multiple animal species.

RESPONSE: *This comment refers to the bullet describing cancer studies in section 2.1. The original text says "Human evidence for cancer are suggestive at best. The epidemiological data are limited by sample size, lack of follow-up, and limited to no exposure data. Several organs in animals were impacted by cancer." ATSDR agrees and text in Chapter 2 were included that tumors were found in multiple animal species, as follows:*

*"**Cancer.** Human evidence for cancer are suggestive at best. The epidemiological data are limited by sample size, lack of participant follow-up, limited to no exposure data, and co-exposure to other carcinogens. Several organs in animals were impacted by cancer and tumors were found in multiple species."*

COMMENT: page 25, line 9: Was it possible to determine why Pliss (1959) found tumors in so many sites in rats? Was the dosage level relatively high? Was a contaminant possibly responsible? Was the strain of test animal particularly susceptible?

RESPONSE: *This comment refers to the third paragraph in section 2.19 (Cancer) and the text that says "Oral exposure to 3,3'-dichlorobenzidine caused tumors in several animal species at several tissue sites. An increased incidence in hepatomas or hepatic tumors has been reported in mice (Osani 1976; Pliss 1959). Tumors were observed in rats at a variety of sites, including the Zymbal gland, mammary gland, bladder, hematopoietic system, skin, ileum, connective tissue, sebaceous glands, salivary gland, liver, thyroid, and papillomas of the bladder (Pliss 1959)." The dosage used appears relatively high as high mortality is reported, 21/50 rats died before the appearance of the first tumors among the surviving rats. Additionally, the development of tumors in multiple locations appeared primarily in 10/22 rats that developed tumors. The text was edited to add in these details as follows:*

"In animal studies, bladder tumors have also been observed in rats and dogs treated with 3,3'-dichlorobenzidine (Pliss 1959; Stula et al. 1975). Oral exposure to 3,3'-dichlorobenzidine caused tumors in several animal species at several tissue sites. An increased incidence in hepatomas or hepatic tumors has been reported in mice (Osani 1976; Pliss 1959). Tumors were observed in rats at a variety of sites, including the Zymbal gland, mammary gland, bladder, hematopoietic system, skin, ileum, sebaceous glands, salivary gland, liver, kidney, thyroid, and papillomas of the bladder (Pliss 1959). Pliss (1959) reported high mortality among exposed rats, and 10/29 rats that developed tumors were responsible for the various locations reported. Stula et al. (1975) administered 3,3'-dichlorobenzidine to 50 male and 50 female ChR-CD rats at a dose of 50 mg/kg/day in the diet. Control groups of 50 males and 50 females were also included in the study. The planned study duration was two years; however the average number of days on the test was 349 days (range of 143-488 days) for females and 353 days (range of 118 to 486) for males. No reason for early mortality was provided. Statistically significant increases in tumor incidences were reported for males: granulocytic leukemia, mammary adenocarcinoma, and zymbal gland carcinoma. The only tumors that showed a statistically significant increase in females were mammary adenocarcinomas (Stula et al. 1975)."

COMMENT: page 25, line 9: It is worth noting that Pliss (1959) and Stula et al. (1975) observed bladder tumors in DCB-treated rats and dogs, in light of the two epidemiology studies in which bladder tumors were found in workers.

RESPONSE: *This comment refers to the third paragraph in section 2.19 (Cancer) and the text that says "Oral exposure to 3,3'-dichlorobenzidine caused tumors in several animal species at several tissue sites. An increased incidence in hepatomas or hepatic tumors has been reported in mice (Osani 1976; Pliss 1959). Tumors were observed in rats at a variety of sites, including the Zymbal gland, mammary gland, bladder, hematopoietic system, skin, ileum, connective tissue, sebaceous glands, salivary gland, liver,*

thyroid, and papillomas of the bladder (Pliss 1959)." ATSDR agrees with the reviewer's recommendation and the following statement was added to begin the following paragraph:

> *"In animal studies, bladder tumors have also been observed in rats and dogs treated with 3,3'-dichlorobenzidine (Pliss 1959; Stula et al. 1975). Oral exposure to 3,3'-dichlorobenzidine caused tumors in several animal species at several tissue sites. An increased incidence in hepatomas or hepatic tumors has been reported in mice (Osani 1976; Pliss 1959). Tumors were observed in rats at a variety of sites, including the Zymbal gland, mammary gland, bladder, hematopoietic system, skin, ileum, sebaceous glands, salivary gland, liver, kidney, thyroid, and papillomas of the bladder (Pliss 1959). Pliss (1959) reported high mortality among exposed rats, and 10/29 rats that developed tumors were responsible for the various locations reported. Stula et al. (1975) administered 3,3'-dichlorobenzidine to 50 male and 50 female ChR-CD rats at a dose of 50 mg/kg/day in the diet. Control groups of 50 males and 50 females were also included in the study. The planned study duration was two years; however the average number of days on the test was 349 days (range of 143-488 days) for females and 353 days (range of 118 to 486) for males. No reason for early mortality was provided. Statistically significant increases in tumor incidences were reported for males: granulocytic leukemia, mammary adenocarcinoma, and zymbal gland carcinoma. The only tumors that showed a statistically significant increase in females were mammary adenocarcinomas (Stula et al. 1975)."*

COMMENT: page 27, line 6: In summation of the in vitro genotoxicity study results, it should be stated that DCB was found to be genotoxic in <u>most</u> of the test systems employed. It is interesting and somewhat unusual that metabolic activation was not required in all the in vitro systems, in view of the need for cytochrome P450-mediated metabolic activation of the parent compound.

RESPONSE: *A statement was added in Section 2.20 Genotoxicity was added summarizing in vitro results as follows: "As summarized in Table 2-3, 3,3'-dichlorobenzidine appeared genotoxic in most in vitro test systems employed."*

COMMENT: page 32, line 16: Are the % absorption values transposed? It seems that a higher % would be unabsorbed from the skin in shorter times following application.

RESPONSE: *The reviewer is referring to the following statement:*

> *"In animals, dermally applied 3,3'-dichlorobenzidine (in acetone) is moderately absorbed. Based on the amount of radioactivity remaining at the site of application, the extent of dermal absorption of applied [14C]-3,3'-dichlorobenzidine to the shaved skin of rats at 1, 8, and 24 hours following the application was estimated to be 6, 23, and 49%, respectively (Shah and Guthrie 1983)."*

The percent absorption rates correspond to the hours of exposure. These percentages indicate that less [14C]-3,3'-dichlorobenzidine is absorbed after only 1 hour of exposure on skin, and more is absorbed after 24 hours of application. No edits have been made in response to the reviewer's comment.

COMMENT: page 32, line 23: Was there information on the quantitative rank order of tissue deposition? The liver would likely attain the highest DCB levels following its ingestion.

RESPONSE: *This comment refers to the section 3.1.2 (Distribution) and the statement "Twenty-four hours after a single oral dose the radioactivity was well distributed with the highest levels of radioactivity found in the liver, kidney, lung, spleen, heart, pancreas, and testes." Currently, tissue deposition is in quantitative rank order. The text was clarified as follows:*

> *"Twenty-four hours after a single oral dose the radioactivity was widely distributed with the highest levels of radioactivity found in the liver, followed by the kidney, lung, spleen, heart, pancreas, and testes. After 96 hours, tissues that retained 0.02% or more of the administered radioactivity were: liver (1.48%), muscle (0.37%), kidney (0.19%), and lung (0.02%)."*

COMMENT: page 32, line 26: It is reasonable to expect that tissue ^{14}C levels would be 3-4 times higher in rats given 6 daily doses than in animals given a single dose, since the terminal half-life is 14 hours. Thus Hsu and Sikka (1982) were justified in concluding that DCB has a fairly low tendency to accumulate in tissues. Also, the propensity for some DCB metabolites to covalently bind to tissue components will prolong retention of radioactivity in the tissues.

RESPONSE: *This comment refers to the statement in section 3.1.2 (Distribution) that says "Repeated administration of [14C]-3,3'-dichlorobenzidine (animals received six daily doses) resulted in tissue radioactive levels three to four times as high as the radioactivity in tissues of animals that received a single dose." Agreed. No edits are suggested by the reviewer.*

COMMENT: page 33, line 8: Tissue distribution patterns also depend upon blood flow. DCB absorbed from the GI tract largely enters the portal venous flow and is taken up by/deposited in/metabolized by the liver. This is not the case of DCM absorbed from different skin surfaces.

RESPONSE: *This comment refers to text in section 3.1.2 (Distribution) that states "The distribution of [14C]-3,3'-dichlorobenzidine in adult male Fisher rat tissues following dermal administration was studied by Shah and Guthrie (1983)." ATSDR agrees with the reviewer's statement and a statement was added to Section 3.1.2 with the reviewer's recommendation as follows:*

> *"The distribution of [14C]-3,3'-dichlorobenzidine in adult male Fisher rat tissues following dermal administration was studied by Shah and Guthrie (1983). Tissues retaining >0.1% of the administered radioactivity 24 hours after application were liver (4.09%), blood (0.75%) and lung (0.45%). The level in the lung was the same at the 8- and 24-hour time points. Differences in the tissue distribution pattern of total radioactivity between the oral and dermal routes of 3,3'-dichlorobenzidine administration may be presumed to reflect differences in the rates of absorption from these sites. Additionally, tissue distribution patterns depend on blood flow, as ingested 3,3'-dichlorobenzidine is absorbed from the gastrointestinal tract and enters portal venous flow, however this would not be expected following dermal exposure. These differences suggest that the target organ in which 3,3'-dichlorobenzidine exerts an adverse effect may depend on the route of exposure to the compound. Organ toxicity can be better evaluated in comparative studies designed to test tissue distribution and persistent exposure."*

COMMENT: page 34, line 19: More recent references should be included here (Iba, 1987, 1989; Wang and Guengerich 2013).

RESPONSE: *This comment refers to section 3.1.3 and the following paragraph:*

"In a 24-hour urine sample of rats given a single dermal application of 3,3'-dichlorobenzidine (50 mg/kg/day), N,N '-diacetyl 3,3'-dichlorobenzidine (but not N-acetyl 3,3'dichlorobenzidine or the unchanged chemical) was detected (Tanaka 1981). Since the mutagenicity of diacetylated product is much less than either the monoacetylated or parent compound (Lazear et al. 1979; Reid et al. 1984; Tanaka 198l), diacetylation may be a detoxification reaction for 3,3'dichlorobenzidine."

The Iba (1987) was added as a reference into section 3.1.3 to support N-acetylation as a pathway for metabolism. Please see previous comments on text from Iba (1989) that were added throughout Chapter 3 of the profile.

COMMENT: page 35, line 6: It would be helpful to include a figure showing the major metabolic pathways involving activation and inactivation. See Zwirner-Baier and Neumann (1998) and Wang and Guengerich (2013) for diagrams.

RESPONSE: *The peer reviewer suggests adding a figure to section 3.1.3 (Metabolism). Studies, including those recommended by the reviewer, were reviewed for potential diagrams showing major metabolic pathways. The discussion of the metabolism of 3,3'-dichlorobenzidine was expanded upon in the profile. As stated in a previous response, the metabolic pathways were not illustrated due to conflicting evidence from various studies. Several figures were added throughout Chapter 3 to better illustrate the discussions.*

COMMENT: page 36, line 4: It should be recognized that these extremely long half-lives are due to/represent <u>covalent binding</u> of radiolabel to the tissues, not the parent compound.

RESPONSE: *This comment refers to the half-lives discussed in the third paragraph in section 3.1.4 (Excretion). ATSDR agrees with the reviewer's comment and text in Section 3.1.4 Excretion was edited as follows:*

> *"Results from animal studies show that 3,3'-dichlorobenzidine administered by gavage is excreted primarily in feces and to a lesser extent in urine. In rats administered a single oral dose of [14C]-3,3'-dichlorobenzidine (40 mg/kg), the elimination from plasma appeared to be biphasic, with half-lives of about 6 and 14 hours for the rapid and slow phases, respectively (Hsu and Sikka 1982). Elimination of 3,3'-dichlorobenzidine-derived radioactivity from liver, kidneys, and lungs also exhibited rapid and slow phases, with half-lives of 5.8 and 77 hours for the liver, 7.1 and 139 hours for the kidneys, and 3.8 and 43.3 hours for the lungs. These longer half-lives are due to the covalent binding of radiolabeled 3,3'-dichlorobenzidine to tissues. Approximately 58-72% of the administered dose was recovered in bile and feces and 23-33% in urine (Hsu and Sikka 1982). Most of the material found in bile and feces consisted of conjugated metabolites, while most of the material in urine consisted of unconjugated metabolites. No detectable residues of 3,3'-dichlorobenzidine were found in urine samples of hamsters administered a single dose of 100 mg/kg purified Yellow 12 (NCTR 1979; Nony et al. 1980). Similarly, 3,3'-dichlorobenzidine was not detected in urine samples of rats fed 3,3'-dichlorobenzidine-derived pigments (C.I.*

Pigment Yellow 12, 16, and 83) in the diet at concentrations of 0.1% (1,000 ppm), 0.3% (3,000 ppm), and 0.9% (9,000 ppm) for 104 weeks (Leuschner 1978)."

COMMENT: page 37, line 6: It is worth pointing out here that bladder cancer was observed in two of six epidemiology studies, as well as in at least two animal species.

RESPONSE: *This comment refers to the text in section 3.1.6 (Animal-to-Human Extrapolations). ATSDR agrees with the reviewer's recommendation, and the text in Section 3.1.6 was edited as follows:*

"Information on the toxicity of 3,3'-dichlorobenzidine for humans and animals is limited, particularly regarding non-cancer end points. Therefore, an attempt to discuss potential interspecies differences or similarities in 3,3'-dichlorobenzidine non-cancer toxicity based on the limited information available is speculative. 3,3'-Dichlorobenzidine is carcinogenic in animals (Osanai 1976; Pliss 1959, 1963; Stula et al. 1975, 1978). While bladder cancer has been observed in occupational studies, there is no conclusive evidence of carcinogenicity of 3,3'-dichlorobenzidine in humans (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004); however, there is concern about occupationally exposed subjects because of 3,3'-dichlorobenzidine's structural similarity with the known human and animal carcinogen benzidine. However, unless a cohort exposed only to 3,3'-dichlorobenzidine is identified and adequate epidemiological studies on such a cohort are conducted, the question will remain unanswered."

COMMENT: page 40, line 7: Should hemoglobin adducts or possible target tissue adducts be considered biomarkers of <u>effect</u>, as well as exposure?

RESPONSE: *This comment refers to section 3.3.1 (Biomarkers of Exposure) and the statement "The detection of hemoglobin adducts as a biomarker of exposure to 3,3'-dichlorobenzidine in rats appears to be a suitable biomarker of exposure for humans." Hemoglobin adducts are only being considered as biomarkers of exposure, not as an effect. There is insufficient evidence to state that DNA adducts extracted from lymphocytes are a biomarker of effect in the ToxProfile.*

COMMENT: page 71, line 15: What is meant by <u>more predictive</u> indicator test systems? What are some examples that would be advocated?

RESPONSE: *This comment refers to the genotoxicity paragraph in section 6.2 (Identification of Data Needs) and the sentence that states "Studies involving more predictive indicator test systems may allow a better assessment of mutagenic potential." The word choice was changed to state additional rather than more predictive as specific assay recommendations are not being made. The following edit was made in Section 6.2 under Genotoxicity:*

"Available studies in animals and in bacterial systems show that 3,3'-dichlorobenzidine did alter genetic material (Ashby and Mohammed 1988; Bratcher and Sikka 1982; Chen et al. 2003, 2014; Chung et al. 2000; Cihak and Vontorkova 1987; Garner et al. 1975; Hering et al. 2018; Iba 1987; Imaoka et al. 1997; Lazear et al. 1979; Makena and Chung 2007; Sasaki et al. 1999; Savard and Josephy 1986; Shiraishi 1986; Wang et al. 2005). Studies involving additional test systems may allow a better assessment of mutagenic potential."