National Toxicology Program Response to the Peer-Review Report

Peer Review of the Draft Report on Carcinogens Monograph on Trichloroethylene

Public Meeting
August 12, 2014

National Institute of Environmental Health Sciences

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Introduction

The NTP convened an *ad hoc* scientific panel ("Panel") to peer review the draft Report on Carcinogens (RoC) Monograph on Trichloroethylene at a public meeting held August 12, 2014, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC (information on the meeting is available at http://ntp.niehs.nih.gov/go/38854). A draft RoC monograph consists of a cancer evaluation component and a substance profile. The Panel had a two-fold charge:

1. To comment on the draft cancer evaluation component for trichloroethylene, specifically, whether it was technically correct and clearly stated, whether the NTP objectively presented and assessed the scientific evidence, and whether the scientific evidence is adequate for applying the RoC listing criteria.

2. To comment on the draft substance profile for trichloroethylene, specifically, whether the scientific justification presented in the substance profile supports the NTP's preliminary policy decision on the RoC listing status of the substance.

The Panel was asked to vote on each of the following for trichloroethylene:

1. Whether the scientific evidence supports the NTP's preliminary conclusion on the level of evidence for carcinogenicity from human cancer studies for each of the three cancer sites: kidney cancer, non-Hodgkin lymphoma (NHL), and liver cancer.

2. Whether the scientific evidence supports the NTP's preliminary listing decision for trichloroethylene in the RoC.

The Panel's peer-review comments were captured in the *Peer Review of the Draft Report on Carcinogens Monograph on Trichloroethylene* ("Peer-Review Report"). Per the process for preparation of the RoC, the NTP prepares a response to the Peer-Review Report and posts it on the RoC website (http://ntp.niehs.nih.gov/go/38854). The NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions and (2) scientific and technical peer-review comments related to identifying scientific issues and improving the technical accuracy, clarity, and objectivity of the monograph.

The NTP carefully reviewed and considered the Peer-Review Report in revising the draft monograph. The revised draft RoC monograph[1] will be shared with the public and the NTP Board of Scientific Counselors (BSC) at their public meeting on December 9-10, 2014, and finalized following the meeting.

[1] Available at http://ntp.niehs.nih.gov/go/37899

Trichloroethylene Peer-Review Panel[2]

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[2] The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists and not as representatives of any organization, company, or governmental agency.

Trichloroethylene Panel Recommendations and NTP Response

NTP's Preliminary Listing Decision for Trichloroethylene in the RoC

The Panel agreed unanimously (9 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list trichloroethylene in the RoC as *known to be a human carcinogen.* This vote was based on epidemiological studies showing sufficient evidence of kidney cancer, together with supporting evidence from toxicokinetic, toxicological, and mechanistic studies. In addition, there is limited evidence of a causal association between exposure to trichloroethylene and non-Hodgkin lymphoma (NHL) from studies in humans. Supporting evidence is provided by studies in experimental animals, which demonstrate that trichloroethylene causes tumors at several tissue sites.

NTP's conclusion regarding the level of evidence for carcinogenicity for kidney cancer

The Panel agreed (8 yes, 0 no, 1 abstention) that the scientific information presented from human kidney cancer studies supports the NTP's preliminary level of evidence conclusion of *sufficient evidence of carcinogenicity.* This conclusion is based on evidence from human epidemiological studies, together with toxicokinetic, toxicological, and mechanistic studies showing a causal relationship between exposure to trichloroethylene and kidney cancer.

NTP's conclusion regarding the level of evidence for carcinogenicity for NHL

The Panel agreed unanimously (9 yes, 0 no, 0 abstentions) that the scientific information presented from NHL studies supports the NTP's preliminary level of evidence conclusion that there is *limited evidence of a causal association between exposure to trichloroethylene and NHL from studies in humans.*

NTP's conclusion regarding the level of evidence for carcinogenicity for kidney cancer

The Panel agreed unanimously (9 yes, 0 no, 0 abstentions) that the scientific information presented from human liver cancer studies supports the NTP's preliminary level of evidence conclusion that the data are *inadequate to evaluate the relationship between liver cancer and exposure to trichloroethylene.*

NTP Response: The NTP concurs with the Panel that trichloroethylene should be listed in the RoC as *known to be a human carcinogen* based on *sufficient evidence of carcinogenicity from studies in humans*.

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed scientific issues related to the cancer hazard evaluation and to improving the clarity and completeness of the Draft RoC Monograph on Trichloroethylene. The specific comments and NTP response to those comments are discussed below and are organized by the type of evidence stream (e.g., human studies, mechanistic studies).

Comments and NTP's response on the human cancer hazard evaluation

Panel Comments:

The Panel raised the following comments, which were mostly related to the initial steps in the cancer hazard evaluation; i.e., study selection, study description, and study quality evaluation (primarily Section 3 Human Cancer Studies and Appendix D).

- Broaden the inclusion/exclusion criteria to include dry cleaning and geographical studies.

NTP Response: Studies of drycleaners and geographical studies were not included in the cancer hazard evaluation because they were not likely to be specific for exposure to trichloroethylene. Moreover, the exclusion of drycleaner studies is consistent with other authoritative reviews. The NTP does not feel that initially including the geographical or drycleaner studies would increase transparency because the reasons for exclusion were clearly noted in the concept document, protocol, and draft monograph, and as noted by the reviewer, the studies would most likely be excluded from the assessment after the study quality evaluation.

- Discuss background information, such as histology/pathology and information on trends of incidence and mortality, for each of the different types of cancer: NHL and its subtypes, and cancers of kidney and liver.
- Provide additional detail on the individual studies (e.g., identify whether trichloroethylene is evaluated as a confounder rather than the substance of interest, and the route of exposure in the drinking water study by Bove *et al.* 2014).[3]
- Discuss the study quality evaluation for some elements in more detail (e.g., selection out of studies, exposure metrics and units, and whether any studies included personal protection equipment in the exposure assessment) and how study quality has changed over time.
- Do not include exposure misclassification as an element in overall evaluation of study sensitivity.
- Clarify how the broad rankings of study quality were reached.

NTP Response: The NTP concurs with these comments and has added the requested information, discussion, and changes requested by the Panel. To address the comment on changes in study quality over time, the NTP added the effect estimates stratified by publication date reported in the meta-analysis by Karami *et al.* (2012)[4]. The NTP has also added a discussion (in Appendix D) on the guidelines used to rank studies by their utility to inform the cancer hazard evaluation and developed new figures (Figure 3.1, 4.1, 5.1, and 6.1) that show how utility rankings for the individual studies met the guidelines. The NTP has clarified that the rankings were for the utility of the study to inform the cancer evaluation (rather than study quality *per se*) and include elements of study quality (e.g., potential biases) and other study elements (e.g., statistical power, length of follow-up).

3 Bove FJ, Ruckart PZ, Maslia M, Larson TC. 2014. Evaluation of mortality among marines and navy personnel exposed to contaminated drinking water at USMC base Camp Lejeune: a retrospective cohort study. Environ Health 13(1): 10.

[4] Karami S, Lan Q, Rothman N, Stewart PA, Lee KM, Vermeulen R, Moore LE. 2012. Occupational trichloroethylene exposure and kidney cancer risk: a meta-analysis. Occup Environ Med 69(12): 858-867.

Other Panel comments were related to later steps in the cancer hazard evaluation, i.e., the interpretation of each individual study's findings and the evaluation of evidence across studies for each cancer site (primarily, Sections 4, 5, and 6 of the monograph).

- Use more consistent language in the interpretation of each individual study's findings in evidence-based tables for the three cancer sites

NTP Response: The language for the conclusions on the studies' findings has been harmonized and individual information on the rationale for the conclusions has been provided

- Provide a systematic review of latency for each of the three cancer sites.

NTP Response: Overall, the database is inadequate to evaluate latency for the three cancer sites because few studies reported effect estimates for each of the cancer sites by categories of time since first exposure or for multiple lagged analyses. The NTP has briefly summarized the overall findings from the studies evaluating latency for each cancer site in the revised monograph.

- Provide more emphasis on the study of cancer risk stratified by glutathione S-transferase theta 1 (GSTT1) genotypes (Moore *et al*. 2010)[5] in reaching the level of evidence conclusion for carcinogenicity from studies in humans.

NTP Response: The NTP concurs with this comment and has revised the monograph accordingly.

Comments and NTP's response on the genotoxicity, mechanistic, and other relevant data

Panel Comments:

The Panel provided the following comments requesting more information on or clarification of the review of the metabolism and genotoxicity data (primarily Sections 1 and 2 of the monograph).

- Discuss the similarity of the glutathione-dependent metabolism data from Kim *et al.* (2009)[6] and Lash *et al*. (2006[7]).
- Clarify that some of the endpoints, such as cellular transformation, reported in Section 2 (Genetic and Related Effect) are not markers of genotoxicity.
- Discuss the relative mutagenicity potency of the different trichloroethylene metabolites.
- Ensure that the information in the text summarizing the genotoxicity is consistent with the findings presented in the appendix and summary tables.

[5] Moore LE, Boffetta P, Karami S, Brennan P, Stewart PS, Hung R, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Gromiec J, Holcatova I, Merino M, Chanock S, Chow WH, Rothman N. 2010. Occupational trichloroethylene exposure and renal carcinoma risk: evidence of genetic susceptibility by reductive metabolism gene variants. *Cancer Res* 70(16): 6527-6536.

[6] Kim S, Kim D, Pollack GM, Collins LB, Rusyn I. 2009a. Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: Formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine. *Toxicol Appl Pharmacol* 238(1): 90-99.

[7] Lash LH, Putt DA, Parker JC. 2006. Metabolism and tissue distribution of orally administered trichloroethylen in male and female rats: identification of glutathione- and cytochrome P450-derived metabolites in liver, kidney, blood and urine. *J Toxicol Environ Health A*, 69(13):1285-1309.

NTP Response: The NTP concurs with these comments and revised the draft monograph accordingly. The NTP has renamed Section 2 to Genotoxicity and Related Effects and clarified which endpoints are not direct markers of genotoxicity. The revised monograph includes a discussion of the requested information.

- Discuss the study by Nestmann *et al.* (1980[8]), which showed that trichloroacetic acid (a metabolite of trichloroethylene) is mutagenic in the Ames test only when dissolved in dimethyl sulfoxide (DMSO). One Panel member raised the possibility that *in vivo* genotoxicity studies and *in vitro* studies using exogenous metabolic activation of trichloroethylene may be false positives if they used DMSO as a solvent.

NTP Response: The NTP has added a discussion of the findings of Nestmann to the revised monograph. It is unlikely that the study showing a false positive with DMSO and trichloroacetic acid affects the overall genotoxicity conclusions because trichloroacetic acid is generally not considered to be a genotoxic metabolite. In addition, studies using trichloroethylene (with metabolic activation) generally used much lower concentrations of DMSO than that needed to cause a false positive (Laque and Ronneberg 1970)[9] and thus the positive genotoxicity findings for trichloroethylene are not likely to have resulted from interactions with DMSO and its metabolite, trichloroacetic acid. These issues have been discussed in the revised monograph.

Several Panel members commented on the interpretation of the mechanistic data for each of the three cancer sites (Sections 4, 5, and 6).

Panel Comments: Kidney cancer (Section 4)

- The Panel felt that the evidence for a mutagenic mode of action was not very strong, although there was evidence of biological plausibility based on both cytotoxicity and genotoxicity as modes of action.
- Findings of the *in vivo* studies should have greater weight than the *in vitro* studies.

NTP Response: The NTP has clarified that the rationale for the listing recommendation is based on human epidemiologic studies with supporting mechanistic data and that the strength of the mechanistic data involves both mutagenicity and cytotoxicity as modes of action. The revised monograph also emphasizes the findings from the *in vivo* genotoxicity studies of trichloroethylene and its glutathione conjugation pathway metabolites.

Panel Comments: NHL (Section 5)

- Conduct a quality assessment of the human studies on immune effects similar to the evaluation of the cancer studies.

NTP Response: The NTP added a discussion of the major strengths and limitations of the immune studies in humans, albeit not to the same level of detail as the study quality assessment conducted for the human cancer studies.

[8] Nestmann ER. Chu I, Kowbel DJ, Matula T. 1980. Short-lived mutation in *Salmonella* produced by reaction of trichloroacetic acid and dimethyl sulphoxide. *Can J Genet Cytol*, 22: 35-40.

[9] Laque WE, Ronneberg CE. 1970. A study of the decarboxylation of trichloroacetic acid in solutions of water and dimethylsulfoxide. *The Ohio Journal of Science,* 70 (2): 97-106.

- The Panel did not agree with the interpretation of several studies on immunosuppression and thought that the monograph's conclusion for trichloroethylene-induced immunosuppression was too strong. They recommended that the section should emphasize immunomodulation rather than immunosuppression.
- Clarify the interpretation of several studies in animals and discuss how differences in species, strain, exposure route, and dose levels might explain the disparity of the immune response in trichloroethylene-exposed animals.

NTP Response: The NTP concurs with the Panel comments and has revised the discussion of trichloroethylene immune effects in Section 5.2 to focus on immunomodulation and has clarified the interpretation of the animal studies and the evidence across studies.

Panel Comments: Liver Cancer (Section 6)

- Discuss the relevance of the importance of proposed mechanisms for liver cancer.
- Summarize additional studies of H-*ras* in liver cancer with exposure to trichloroethylene vs. trichloroacetic acid vs. dichloroacetic acid.

NTP Response: The monograph was revised to indicate which modes of action had more limited support and to include additional studies (Eastmond 2012[10], Ferreira-Gonzalez *et al.* 1995[11], Anna *et al.* 1994[12]) on trichloroethylene metabolites, H-*ras* mutations, and liver cancer.

Comments and NTP's response on exposure information in the draft substance profile

Panel comments:

- Provide a description of changing patterns of trichloroethylene use, including decreased use for metal degreasing, reduced emission based on evidence from EPA's Toxics Release Inventory, and decreased detection of trichloroethylene blood levels reported in the most recent National Health and Nutrition Examination Survey (NHANES).
- Provide information on the potential for dermal exposure to trichloroethylene in water during showering.

NTP Response: The NTP concurs with these comments and has revised the monograph accordingly.

[10] Eastmond DA. 2012. Factors influencing mutagenic mode of action determinations of regulatory and advisory agencies. *Mutation Research* 751:49-63.

[11] Ferreira-Gonzalez A, DeAngelo AB, Nasim S, Garrett CT. 1995. *Ras* oncogene activation during hepatocarcinogenesis in B6C3F1 male mice by dichloroacetic and trichloroacetic acids. *Carcinogenesis* 16(3):495-500

[12] Anna CH, Maronpot RR, Pereira MA, Foley JF, Malarkey DE, Anderson MW. 1994. *Ras* proto-oncogene activation in dichloroacetic acid-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. *Carcinogenesis* 15(10):2255-2261.