National Toxicology Program

Peer Review of Draft Report on Carcinogens (RoC) Monograph on Trichloroethylene

August 12, 2014

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

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I. Attendees

Peer Review Panel

David Eastmond (Chair), University of California, Riverside
Sarah Blossom, University of Arkansas for Medical Sciences
Kenneth Cantor, KP Cantor Environmental, LLC
John Cullen, North Carolina State University
George Douglas, George R. Douglas Consulting
S. Katherine Hammond, University of California, Berkeley
Lawrence Lash, Wayne State University
Marie-Elise Parent, Université du Québec
David Richardson, University of North Carolina, Chapel Hill
Paolo Vineis, Imperial College, London

National Toxicology Program Board of Scientific Counselors Liaison

Dale Hattis, Clark University (by telephone)

Other Federal Agency Staff

H. Edward Murray, Agency for Toxic Substances and Disease Registry
Sharon Silver, National Institute for Occupational Safety and Health

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Neela Guha, International Agency for Research on Cancer (IARC, by telephone)

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Public Attendees

James Bus, Exponent
Caffey Norman, Squire Patton Boggs (by telephone)
Lorenz Rhomberg, Gradient Corporation (by telephone)

Webcast

Christopher Bevan, CJB Consulting, LLC
Jack Bishop, NIEHS
John Bell, Halogenated Solvents Industry Alliance (HSIA).
Lawrence Stilwell Betts. Eastern Virginia Medical School
Lorenz Rhomberg, Gradient
Lynn Pottenger, The Dow Chemical Company
Resh Putzrath, U.S. Navy
Sangeeta Khare, U.S. Food and Drug Administration
Seung-Tae Chung, National Institution of Food and Drug Safety, Korea
Whitney Arroyave, Social and Scientific Systems

II. Welcome and Introductions

The National Toxicology Program (NTP) Peer Review Panel for the Draft Report on Carcinogens (RoC) Monograph on Trichloroethylene convened on August 12 in Rodbell Auditorium, Rall Building, National Institute for Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. David Eastmond served as chair. Dr. Dale Hattis participated by telephone as the NTP Board of Scientific Counselors (BSC) liaison. Dr. Sharon Silver attended representing the National Institute for Occupational Safety and Health (NIOSH). Representing the NTP were Dr. Linda Birnbaum, NTP Director; Dr. John Bucher, NTP Associate Director; Dr. Mary Wolfe, Deputy Division Director for Policy; and Dr. Ruth Lunn, Director, Office of the RoC. Dr. Lori White, Staff Scientist, Office of Liaison, Policy and Review, served as the Designated Federal Official.

Dr. Eastmond called the meeting to order at 8:35 a.m., welcomed everyone to the meeting, and asked all attendees to introduce themselves. Dr. Bucher welcomed and thanked the attendees. Dr. White read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. Eastmond briefed the panel and the audience on the format for the peer review.

III. Process for Preparing the Draft RoC Monograph

III.A. Presentation

Dr. Ruth Lunn, NIEHS, presented background information on the RoC and on the process and methods used to prepare the draft RoC monograph on trichloroethylene. She noted that the RoC is congressionally mandated and identifies substances that pose a cancer hazard for U.S. residents. It is prepared for the Secretary of Health and Human Services (HHS) by the NTP and is cumulative, including the profiles for newly listed substances and for all substances listed in previous reports.

The rationale for selecting trichloroethylene as a candidate substance for the RoC was that occupational exposure to trichloroethylene occurs and that it is ubiquitous in the environment. Trichloroethylene is currently listed in the RoC as *reasonably anticipated*

to be a human carcinogen and an adequate database of human cancer studies of trichloroethylene has been published since the last RoC review in 2000.

Dr. Lunn noted that for every substance proposed for review, a concept document is written that explains the rationale and proposed approach for the review. Once a substance is formally selected for review, a protocol for preparing the draft monograph is prepared, and the draft RoC monograph is written. The draft monograph consists of two parts: (1) a literature-based cancer evaluation and (2) the draft substance profile, which contains the preliminary listing recommendation and a summary of the scientific evidence considered to be key for reaching the recommendation.

The process for preparing the RoC consists of the following parts: (1) nomination and selection of candidate substances, (2) scientific evaluation of the candidate substances, (3) public release and peer review of the draft monographs, and (4) submission of the substance profiles to the Secretary of HHS for approval. The process provides opportunities for public comment, scientific input, and peer review of the scientific information. Public comments were received on the nomination of trichloroethylene and on the draft monograph.

Dr. Lunn outlined the steps of the review process that had been completed for scientific evaluation of trichloroethylene and preparation of the draft monograph. In addition, she noted additional opportunities for obtaining scientific and/or public inputs. On March 17, 2014, a public webinar was held on methods for assessing trichloroethylene exposure and cancer outcomes. On April 3, 2014, ORoC convened a scientific informational group to evaluate whether trichloroethylene-induced immune effects could be helpful in explaining possible mechanisms of non-Hodgkin lymphoma (NHL). The decision to focus the draft monograph on three tissue sites — kidney, NHL, and liver — was based on authoritative reviews and on tissue-site concordance with cancer in experimental animals. No new studies were identified that would change the previous conclusion that there is sufficient evidence of carcinogenicity from cancer studies in experimental animals. Therefore, the draft monograph considers findings from animal cancer studies only in the discussion of mechanisms of carcinogenicity. The exposure information was updated in the substance profile but is not discussed elsewhere in the draft monograph. The discussion of mechanistic and other data used information from several authoritative reviews supplemented by information from recent or key studies.

Dr. Lunn outlined the structure of the draft monograph, which was organized by cancer site. She also reviewed the literature search strategy and the criteria used to assess study quality, the level of evidence for carcinogenicity, and the mechanistic data. She then reviewed the RoC criteria for *sufficient* or *limited evidence of carcinogenicity* from studies in humans (epidemiological or clinical studies or studies of human tissues or cells) and the RoC criteria for listing of substances as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen.*

The charge to the Peer Review Panel was:

- To comment on the draft cancer evaluation component, specifically, whether it is technically correct and clearly stated, whether the NTP has objectively presented

and assessed the scientific evidence, and whether the scientific evidence is adequate for applying the listing criteria.

- To comment on the draft substance profile, specifically, whether the scientific evidence supports the NTP's preliminary RoC listing decision for the substance.

The panel would be asked to vote on the following questions:

- Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from cancer studies in humans for each tissue site.
- Whether the scientific evidence supports the NTP's preliminary policy decision on the RoC listing status of the substance.

Dr. Lunn noted that the draft monograph would be revised based on NTP's review of the peer-review comments. Additional steps in the process after the peer review meeting were highlighted. The panel's comments are captured in this peer-review report, to which the NTP will write a response explaining how the comments were considered in revising the monograph. The revised monograph, peer-review report, public comments, and NTP response will be provided to the BSC, after which the monograph will be finalized. Once all reviews have been completed for the next edition of the RoC, the substance profiles for newly reviewed candidate substances will be submitted to the Secretary, HHS, for approval or disapproval, and the next edition of the RoC will be published.

IV. Public Comments

IV.A. Oral Public Comments

IV.A.1 The Halogenated Solvents Industry Alliance

Mr. Caffey Norman of Squire Patton Boggs commented by telephone on behalf the Halogenated Solvents Industry Alliance (HSIA). He stated that the 30 days allowed for public review of the monograph was inadequate for meaningful input. He expressed concern that the draft monograph had not been structured through the use of an organized data integration framework and that the decision to change the listing of trichloroethylene appeared to rest on the NTP's evaluation of the available data on kidney cancer. He thought the draft monograph applies unbalanced interpretation of the data from epidemiological and toxicity studies to generate unfounded concerns about the effects of trichloroethylene exposure on the kidney. The epidemiological data are too weak to be considered sufficient; the animal cancer bioassay data are equivocal and inconsistent; trichloroethylene genotoxicity is probably significant, if at all, only after nephrotoxicity has occurred; and there is little support for glutathione conjugation as a relevant metabolic pathway. The toxicokinetic, toxicological, and mechanistic data for trichloroethylene do not add support for the designation as a known human carcinogen.

Mr. Norman emphasized that an overall weight-of-evidence analysis of the epidemiologic research does not support the conclusion that there is *sufficient evidence of a causal association* between human exposure to trichloroethylene and kidney cancer. The draft monograph relies on three meta-analyses to provide additional

statistical power to the evaluation of often-disparate study results. However, meta-analysis of epidemiological data cannot establish a causal relationship (Weed, 2010). Moreover, even if the meta-analyses are accepted at face value, the summary relative risks (ranging from 1.2 to 1.4) only suggest a weak association and cannot support a *known human carcinogen* classification, which requires a causal relationship. It is well established that relative risks less than 2 are not sufficient evidence of causation.

Mr. Norman noted that the National Academy of Sciences (NAS) evaluation of health outcomes of exposure to trichloroethylene in contaminated water supplies at Camp Lejeune, NC, concluded that there was only *limited/suggestive evidence of an association* between trichloroethylene exposure and kidney cancer (NAS, 2009). The draft monograph does not discuss or explain why the NTP's conclusion differs from that of the NAS.

Mr. Norman concluded by stating that under the listing criteria, the evidence for the carcinogenicity of trichloroethylene in humans at most supports the *reasonably anticipated* classification.

IV.A.2 Gradient Corporation

Dr. Lorenz Rhomberg of Gradient Corporation commented by telephone on behalf of the Dow Chemical Company. He disclosed that he had previously worked with the HSIA on the issue of whether trichloroethylene causes cancer.

Dr. Rhomberg noted that for complex chemicals like trichloroethylene, it is important to fully explore the metabolic differences across species, the role of precursor tissue toxicity, and possible modes of action. He said it is important to integrate this information into the whole body of evidence, rather than reaching conclusions based on separate evaluations of the epidemiological, animal, and mechanistic data. Information about mode of action and dose-response patterns in animal studies should provide a basis for determining whether the epidemiology studies provide evidence for a causal process. It is important to account for the inconsistency of the epidemiology results for trichloroethylene and the lack of a dose response.

Dr. Rhomberg noted that in rats, kidney cancer was observed only as a rare tumor at a high and toxic dose of trichloroethylene; that metabolic delivery of that dose is probably not possible in humans; and that genotoxicity of some metabolites was observed only *in vitro,* and not in *in vivo,* because of metabolic nonlinearity. These issues were not fully addressed in the draft monograph, particularly in the final evaluation of causality, and the conclusion of *sufficient evidence* relies on the epidemiology. Dr. Rhomberg emphasized the importance of looking at alternative explanations for associations observed in epidemiological studies and of showing an underlying causal basis for carcinogenicity. Even for a hazard assessment, dose-response patterns are informative about modes of action and causality. Dr. Rhomberg concluded by stating that if a substance is to be called a *known carcinogen,* it is important to integrate the evidence and frame an argument for causality.

IV.B. Scientific Issues in Written Public Comments

Dr. Wolfe, NIEHS, reported that the NTP had received one written public comment on the draft monograph from Ms. Faye Graul, Executive Director of HSIA. Dr. Wolfe summarized the major scientific issues raised to support the HSIA's overall conclusion that there is a compelling case against changing the RoC listing recommendation from *reasonably anticipated* to *known to be a human carcinogen.*

According to HSIA, the epidemiological evidence for an association between trichloroethylene and kidney cancer is weak and not sufficient. The NTP's assessment of the epidemiological data conflicts with the NAS report from Camp Lejeune, which concluded that the evidence for an association between trichloroethylene and kidney cancer was "limited or suggestive." The overall weight-of-evidence analysis of the epidemiological data does not support a conclusion of *sufficient evidence of a causal association*. Limitations of the studies include weak associations, potential for confounding, and exposure uncertainty. The meta relative risks cannot support classification as a *known human carcinogen,* because meta-analysis is not a tool for establishing a causal relationship. Also, the meta relative risks are between 1.2 and 1.4, and relative risks less than 2 are not sufficient to establish causation.

The data from studies of kidney cancer in rodents are equivocal and inconsistent. The renal tumor incidence was low in all three NTP studies, despite exposure to doses at or exceeding the maximum tolerated dose. Many of the studies had methodological problems. The authors of the 1988 NTP (NTP, 1988) study considered it to be an inadequate study of carcinogenicity, and the oral and inhalation studies by Maltoni *et al.* (1986, 1988) are considered controversial and used nonstandard methodology.

The toxicologic and mechanistic data also do not support a *known human carcinogen* classification. The presumed mode of action via glutathione conjugation is based on flawed research. Research from three laboratories indicated that metabolism of trichloroethylene via this pathway is very low, and lower in humans than in rodents. Kidney toxicity in rodents cannot be explained solely by the extent of dichlorovinyl cysteine (DCVC) production and activation. Kidney damage in humans is highly unlikely to occur at occupational exposure levels and is of no concern for the general population. Genotoxicity is not a likely mechanism for kidney carcinogenicity, because DCVC is weakly genotoxic *in vivo,* and only low levels are produced; DCVC did not induce tumors in rats under a protocol expected to show tumor induction by a genotoxic mode of action; and although DCVC activation is greater in mouse kidney than in rat kidney, trichloroethylene has not induced kidney tumors in mice.

Dr. Wolfe asked the panel to consider HSIA's comments in its review and noted HSIA's public comment was posted to the NTP website.

V. Peer Review of Draft RoC Monograph on Trichloroethylene

V.A. Cancer Evaluation Component

V.A.1 ADME, Toxicokinetics, and Genetic Effects

V.A.1.1 Presentation

Mr. Stanley Atwood, ILS, presented an overview of the key information in the draft monograph sections on disposition and metabolism of trichloroethylene and genetic and related effects of trichloroethylene and its metabolites. Trichloroethylene is a small lipophilic molecule, well absorbed via all routes of exposure and rapidly distributed to all tissues. Under most exposure circumstances, the majority of trichloroethylene is excreted as metabolites in urine. Two metabolic pathways have been identified that are common to all mammalian species studied, and trichloroethylene metabolism is qualitatively similar in rodents and humans.

The dominant pathway is oxidative metabolism by cytochrome P450 (primarily CYP2E1) metabolism, primarily in the liver, producing a number of stable urinary metabolites, some of which are used as biomarkers of exposure. The main pathway is through chlorine migration to form chloral and chloral hydrate, which are reduced by alcohol dehydrogenase to form trichloroethanol (TCOH), which is further oxidized by P450 to form trichloroacetic acid (TCA).

The other key pathway is glutathione conjugation, which produces many reactive metabolites, particularly in the kidney. The initial step is formation of dichlorovinyl glutathione (DCVG), mainly in the liver, but also in the kidney and lung, depending on the route of exposure. Subsequent metabolism of DCVG occurs largely in the kidney, to form DCVC, which is primarily deactivated to form the only observed stable urinary metabolite of the glutathione pathway, *N*-acetyl dichlorovinyl cysteine (NAcDCVC). DCVC is also metabolized by renal cysteine conjugate β-lyase or flavin-containing monooxygenase 3 to form reactive metabolites. The glutathione pathway is believed to important, particularly for kidney toxicity. Flux in the glutathione pathway is uncertain, but is variable and may be higher than previously thought. In humans, it is affected by genetic polymorphisms in metabolizing enzymes and by exposure to P450 inducers or inhibitors, the impact of which is likely greater at high substrate concentrations. The U.S. Environmental Protection Agency's (EPA's) physiologically based pharmacokinetic model indicates that glutathione metabolism of trichloroethylene could exceed oxidative metabolism at high exposure levels (1,000 ppm via inhalation or 1,000 mg/kg body weight per day via oral exposure).

Trichloroethylene and its metabolites induce genetic and related effects *in vitro*, primarily DNA strand breaks and chromosome damage. The effects of trichloroethylene have been attributed to the presence of metabolites or chemical stabilizers. Of the oxidative metabolites, chloral hydrate showed the highest genotoxic potential (at high doses). Effects of DCVG or DCVC include mutations and DNA and chromosome damage. With DCVC, mutations were decreased by a β-yase inhibitor and increased by kidney-derived metabolic activation. Although limited, the *in vivo* studies showed similar effects, including DNA strand breaks in kidney tissue; however, the results for a number

of end points were mixed. Mr. Atwood noted that the NTP considered DCVC to be the most potent of the metabolites in the *in vitro* genotoxicity assays.

V.A.1.2 Peer Review Comments

Dr. Lawrence Lash, first reviewer, stated that the discussion on metabolism was clear, succinct, and appropriately focused on the key aspects of the topic. In particular, the monograph included a good discussion of estimating flux through the metabolic pathways and the concentration-related differences in relative flux between the oxidative and glutathione pathways. In response to the HSIA's comment and also reported in the draft monograph, that three sets of studies had found much lower levels of trichloroethylene metabolism via glutathione conjugation than were found by Dr. Lash's laboratory, Dr. Lash said that this was inaccurate. In fact, the levels found by Kim *et al.* (2009) were in close agreement with those found by Dr. Lash. Dr. Lash found the section on genotoxicity to be a good, concise summary. He suggested adding a discussion of relative potency among the mutagenic metabolites of trichloroethylene (citing Moore and Harrington-Brock, 2000), as this is important for understanding the mechanism.

Dr. George Douglas, second reviewer, noted that TCA is mutagenic in the Ames test only when dissolved in dimethyl sulfoxide (DMSO), as shown by Nestmann *et al.* (1980), raising the possibility of false-positive results in the *in vitro* tests that used DMSO as a solvent. False-positive results could also have occurred in *in vitro* tests of trichloroethylene with metabolic activation. It is not known whether DCA reacts similarly with DMSO or whether the use of DMSO as a vehicle in *in vivo* studies could similarly cause false-positive results. The interaction of TCA with DMSO is a possible explanation for the variability of the *in vitro* genotoxicity results. This issue, as well as the issue of pH in testing of TCA, needs to be considered in evaluating the genotoxicity results.

Dr. Douglas objected to the lumping of cell transformation and protein adduct formation with genetic effects, noting that protein adducts are indicators of exposure and cellular transformation may not be a genotoxic effect, at least directly. DNA adduct formation and sister-chromatid exchanges also are indicators of exposure, not genetic effects per se, as DNA adducts do not result in mutation without cell turnover, and sister-chromatid exchanges are reciprocal events. These concerns could affect interpretation of the data, as could consideration of relative potencies (i.e., whether an effect seen *in vitro* could be elicited by the concentrations of trichloroethylene or its metabolites observed in the target tissue). Dr. Douglas noted a problem with the references for the second paragraph of Section 2.7.4, some of which are incorrect or missing from the reference list. He also noted that the genotoxicity data for trichloroethylene metabolites are not included in Appendix C.

Dr. Douglas and Mr. Atwood provided further information on the observations of Nestmann *et al.* No mutagenic by-products of the reaction of TCA with DMSO have been characterized. DMSO accepts a proton from TCA, resulting in formation of chloroform (which is not mutagenic) and a dramatic transitory increase in pH. The mutagenic effect observed is probably related to the increase in pH. Mr. Atwood noted

that the effect depends on the concentration of DMSO used. He also noted that the draft monograph did not propose genotoxicity of oxidative metabolites in the liver as a mode of action for trichloroethylene carcinogenicity, and that this issue does not apply to the genotoxicity results for the glutathione-pathway metabolites. Dr. Eastmond noted that DMSO also inhibits CYP2E1.

Dr. John Cullen, third reviewer, said the discussion in the draft monograph was well written, clear, and thorough. The overall evaluation of the studies was well done, identifying the individual studies' potential problems and strengths. He agreed with Dr. Douglas that the DMSO issue should be pursued and that protein adduct formation is not relevant. He suggested that at toxic doses of trichloroethylene, the combination of DNA adduct formation and toxicity-induced cell proliferation might provide an alternative explanation for tumor production, and that this should be discussed.

V.A.1.3 Panel Discussion

Dr. Eastmond commented that the genotoxic effects observed for TCA tend to disappear when the acid is neutralized, and suggested that the monograph's discussion of the issue of acidity be strengthened. He found the descriptions of the genotoxicity results in the text to be inconsistent with the summary in Table 2-1. Because most organic solvents used in *in vitro* genotoxicity tests can potentially interfere with CYP2E1 bioactivation, negative results *in vitro* should be interpreted with caution. In the metabolic pathway diagrams, multiple arrows should be used to indicate where multiple enzymatic steps take place. Dr. Eastmond also suggested that since Section 2 covers genetic *and related* effects, discussion of protein adducts and cell transformation was appropriate, though the NTP should consider whether to break these out separately in the summary table.

Dr. Douglas agreed with Dr. Eastmond about the inconsistency between Table 2-1 and the text; in particular, he could not find a source in the text for the positive results for TCA for chromosomal aberrations *in vivo.* He suggested including key references in the summary table. He also stated that if DCVC is an important metabolite and causes gene mutations *in vitro;* accordingly, the negative results for gene mutation following trichloroethylene exposure *in vivo* (Douglas *et al.,* 1999) are hard to explain.

V.A.2 Human Cancer Studies Overview

V.A.2.1 Presentation

Dr. Jennifer Ratcliffe, ILS, presented an overview of human cancer study selection and quality evaluation. Studies were included if they considered the kidney cancer, NHL, and/or liver cancer, provided trichloroethylene-specific risk estimates, and were peer reviewed. Studies of dry cleaners were excluded because trichloroethylene exposures were low and mixed, and most ecological studies were excluded because of mixed and poorly characterized exposures and the lack of trichloroethylene-specific risk estimates.

The selected studies included 16 cohort and nested case-control studies, categorized as 3 Nordic occupational cohort studies, 5 U.S. aerospace/aircraft worker cohort studies, 7 other assorted industry cohort and nested case-control studies, and a U.S.

drinking-water-exposure cohort study (the Camp Lejeune study), and 15 case-control studies, categorized as 7 studies on kidney or liver cancer and 8 studies on NHL and related lymphoma subtypes. The studies were evaluated according to guidelines laid out in the protocol and with input from the public webinar and technical advisors. They were evaluated for likelihood of selection and attrition bias, quality of the exposure assessment, likelihood of exposure misclassification, study sensitivity (ability to detect an effect, based on statistical power, exposure levels and duration, length and completeness of follow-up, and potential for exposure misclassification), quality of disease assessment, and methods for evaluating potential confounding. These evaluations were used to rank overall study quality in broad categories.

Overall strengths of the database were that it was large, included studies of different occupations in different geographical locations, and included several high or moderate quality cohort and case-control studies of kidney cancer and NHL. Some studies in specific industries controlled for co-exposures, and many of the case-control studies controlled for lifestyle factors. Limitations of the database were inclusion of some studies considered low to moderate quality because of low sensitivity and a few studies with biases or potential confounding towards an overestimate of risk. Few studies had data adequate for evaluation of exposure-response relationships.

V.A.2.2 Peer Review Comments

Dr. Kenneth Cantor, first reviewer, thought the overall approach was quite reasonable, and had no specific comments on the overview.

Dr. Katherine Hammond, second reviewer, approved of the rigor of the approach and considered the detailed analysis of the impact of exposure-assessment quality to be excellent. She agreed with NTP's conclusions that most of the studies had limited ability to address exposure-response relationships, that the potential for misclassification was generally non-differential and would most likely bias towards the null, and that misclassification between exposure groups in subgroup analyses would most likely attenuate any exposure-response relationships. However, the discussion of the different exposure metrics used in the studies should be clearer and integrated, the units of measurement should be standardized, and the use of tables for comparison of exposure data should be considered. Dr. Hammond also noted that it is inaccurate to refer to exposure duration or exposure probability as "surrogates" for exposure intensity. She suggested more discussion of the wide variation in the quality of the job exposure matrices, of the strengths and limitations of biomonitoring with urinary TCA, and of the high variability of true exposure in occupational settings.

Dr. David Richardson, third reviewer, said the approach to identification of the literature and extraction of the information seemed reasonable and that the attempt to structure the description of the study characteristics was useful. His noted that his comments were primarily suggestions for future reviews. Although he saw the point of excluding categories of studies less likely to be informative, such exclusions could appear to be *ad hoc* or arbitrary. He suggested a more comprehensive approach, including a wider range of studies in the review, and then ranking them as more or less informative. He commented that evaluation of study quality was defined more clearly in Dr. Ratcliffe's

presentation than in the draft monograph. He found the discussion of information bias to be particularly well written and nuanced. He had concerns about including study sensitivity category as a study quality element because it conflates statistical power with exposure misclassification. Statistical power is reflected in the confidence intervals and in the forest plots. It is possible to have high-quality studies aimed at estimating low-magnitude exposure effects. The exposure magnitudes are important to consider with the magnitudes of the effect estimates, such as was done in the plots of the rank-ordering of studies by exposure level in the cancer evaluations. Dr. Richardson agreed with Dr. Hammond that the different types of exposure assessment and their strengths and weaknesses should be discussed. He also suggested organizing consideration of the studies by the trajectory of the science and the quality of the literature (which has improved over time), rather than by industry. He suggested distinguishing between studies in which trichloroethylene was or was not the primary exposure of interest, which had implications for variable selection and analysis and interpretation of the results.

Dr. Marie-Elise Parent, fourth reviewer, was impressed by the level of detail, accuracy, and completeness of the information. The important criteria for evaluation of study quality were considered, and the arguments were balanced. However, for consideration of study sensitivity, she found the Appendix D tables more useful than the text, and would have liked to see a table rating the 16 cohort studies with respect to quality of exposure assessment. She also found it difficult to relate the discussion in the text to the table evaluating overall study quality; it was not clear how all of the various criteria had been integrated to assign the rankings. It would be useful to have a table rating each study on each of the criteria. Dr. Parent suggested discussing whether protective equipment had been used by workers or was taken into account in the exposure assessments, and she wondered whether toxicological information could shed light on what past exposure periods would be most important in lagged analyses. In response to comments from the HSIA, Dr. Parent disagreed that the assessment gave too much weight to the meta-analyses; she noted that the NTP reached different conclusions for kidney and liver cancer despite very similar meta-analysis results. Regarding the request that the NTP’s analysis be compared with that of the NAS, she noted that a balanced request would have asked for comparison with analyses by other agencies as well, such as IARC, whose analysis is concordant with that of the NTP.

V.A.2.3 Panel Discussion

Dr. Paolo Vineis found the ranking of studies by quality useful, but agreed about the need for greater transparency in the reasoning that led from the evaluation of bias in the individual studies to the overall ranking, particularly the weight assigned to each type of bias. It might be useful to go beyond simply presenting forest plots to addressing study heterogeneity and publication bias. It would also be useful to have more background information on the different types of cancer, including geographic variation and time trends, and their impact on estimation of associations with trichloroethylene exposure. Dr. Vineis disagreed with the public comments that the evaluation of kidney cancer was based solely on the meta-analyses, noting that the meta-analyses were considered in the context of an original evaluation of the literature.

Dr. Neela Guha, NIOSH, in response to the public comments, she noted that the most recent meta-analysis reported in the draft monograph was in fact conducted according to recommendations from the NAS. She also noted that the NTP used the meta-analyses as a tool to assess the overall data and study heterogeneity. Dr. Silver said it would be helpful for the tables to systematically address latency.

V.A.3 Kidney Cancer

V.A.3.1 Presentation on Human Studies

Dr. Lunn presented an overview of the key information in the draft monograph section on human kidney cancer studies. Because kidney cancer is relatively rare and has a high survival rate, studies based on incidence are more informative than those based on mortality. The main, potentially confounding co-exposure is tobacco smoking. Twelve cohort or nested case-control studies, seven case-control studies, and two meta-analyses were reviewed. The most informative studies were a 2005 cohort study of U.S. aerospace workers, a 2009 case-control study of French screw-cutting workers, and a 2010 multi-center case-control study in Central and Eastern Europe. Most of the studies ranked as being of low or low/moderate quality had limited sensitivity to detect an association, and two studies had potential biases that would likely lead to overestimation of risk.

The NTP concluded that there is credible evidence of an association between increased kidney cancer risk and exposure to trichloroethylene based on (1) consistent findings of increased risk across the studies of different study designs, in different geographical locations, and in different occupational settings; (2) evidence of increasing risk with increasing level or duration of exposure; (3) meta-analyses showing statistically significant increased risk across studies; and (4) the fact that the findings were unlikely to be explained by chance, bias, or confounding.

A forest plot of relative risk estimates for the highest exposure group in each study (based on intensity, cumulative, duration, or surrogates of exposure) showed a trend for higher risks in the moderate- to high-quality studies than in the low- to low/moderate-quality studies, with the highest risks in the two studies with potential for positive bias. The latter two studies had both high exposure levels and high-risk estimates. Although the magnitude of the risk estimate may have been overestimated, the potential for bias probably did not negate the findings of increased risk, and, the overall conclusion of increased risk was the same whether or not these two studies were included. The three most informative studies showed a trend towards increasing risk with increasing cumulative exposure category.

In a forest plot showing relative risk across studies by three broad categories of estimated exposure level, elevated risks were found for most studies in the moderate and high exposure categories with the highest risk found in studies with the highest exposure category; however, the comparison was limited by the studies' use of different exposure metrics. The two meta-analyses found similar statistically significant increased risks, with no evidence of publication bias or heterogeneity; neither meta-analysis included the two studies with potentially biases towards an overestimation of the effect

estimate. The meta relative risks were robust and not sensitive to removal of individual studies or selection of alternative risk estimates. In addition, there was no evidence of publication bias or heterogeneity. Confounding by co-exposures was unlikely to account for the increased risk, because studies of specific industries controlled for known co-exposures, and in the studies of diverse occupations with varied types and patterns of co-exposures, the prevalence any specific co-exposure was likely to be low. In the cohort studies, the absence of an association between trichloroethylene exposure and lung cancer argued against confounding by smoking, which is a weak risk factor for kidney cancer, and most of the case-control studies controlled for smoking. A meta-analysis of trichloroethylene exposure and lung cancer found a relative risk of approximately 1. Potential biases were unlikely to explain all of the excess risk of kidney cancer.

V.A.3.2 Peer Review Comments on Human Studies

Dr. Richardson, first reviewer, found the review to be very useful. He suggested clarifying which specific kidney cancer outcomes (renal cell carcinoma, all kidney cancer) were being considered, both as end points in the studies and for potential confounders. He agreed that weighting of the study quality categories in the overall ranking was sometimes unclear. He found it surprising that potential confounding by smoking could be ruled out across the studies and suggested distinguishing between the population based case-control studies that controlled for smoking and the cohort mortality studies for which smoking history information was not available. The discussion of selection bias could be more nuanced, especially concerning participation rates and self-selection into or out of studies. Also, selection bias in occupational studies is not just initial but related to ongoing participation in the workforce. Despite these concerns, Dr. Richardson agreed with the study quality evaluation.

Dr. Parent, second reviewer, thought the studies on kidney cancer were well synthesized and interpreted, and agreed with the extraction of relative risks where several were available in the original articles. She was uncomfortable with the statement that meta-analyses can increase the statistical power of underpowered studies; rather, their aim is to synthesize the available data and investigate reasons for heterogeneity. She also suggested harmonizing the wording used in the tables to summarize study characteristics and eliminate differences in wording that did not convey meaningful differences in study characteristics.

Dr. Cantor, third reviewer, said more emphasis should be placed on the effects of gene polymorphisms on risk, particularly differences between individuals positive and null for glutathione S-transferase theta 1 (GSTT1). He questioned whether discussion of this issue belonged in the section on genotoxicity. The basis for the order in which studies were listed in Appendix D was not clear. Dr. Cantor agreed with the need for more background information on cancer types, at least for kidney cancer and NHL, including the proportions of cancers of different cell types, because their etiologies differ. In response to the public comments, he noted almost all of the studies reviewed in the draft monograph were published after the NAS report from Camp Lejeune, reducing its relevance, and that the Camp Lejeune study was limited by being a mortality study of a young population, with only 11 cases in the highest exposure group.

Dr. Hammond, fourth reviewer, noted that the Camp Lejeune study was the only one that looked at exposure by ingestion, rather than inhalation. She suggested more discussion of why this study was considered to be relatively uninformative. She said the assessment of exposure in the epidemiological studies was well done, and a table summarizing exposure levels would be helpful. An explanation of the logic behind the assignment of exposure categories in the forest plot as well as actual exposure concentrations or ranges would also be helpful. In response to the public comments, she noted that it is not generally accepted that relative risks under 2 are not informative. She noted that the more detailed exposure information provided in Appendix D reinforced the association of higher exposure levels with higher relative risks across studies. Moore *et al.* (2010), who used several different exposure metrics and dichotomized exposure groups, found significantly elevated risks in the higher exposure groups for all metrics. Moore *et al.* reported that in almost all cases, trichloroethylene exposure had occurred at least 20 years before the onset of disease. Dr. Hammond noted the importance of considering how to evaluate lagging; an apparent long latency could also be due to reduction in exposure levels over time. Her overall conclusion was that several studies made a very convincing case that trichloroethylene is a carcinogen in humans.

Dr. Lunn clarified that the tables on study sensitivity and exposure-response analysis in Appendix D included all exposure information for each study available from any source, followed by the exposure category to which the study was assigned. She noted that in the case-control studies, all kidney cancers were renal-cell carcinoma, and that in the cohort studies the International Classification of Diseases codes usually included other types of kidney cancer. With respect to improvements in study quality over time, Dr. Lunn thought that one of the meta-analyses had stratified by age of the studies and had found a slightly higher relative risk for the newer studies.

V.A.3.3 Panel Discussion of Human Studies

Dr. Vineis suggested adding a paragraph discussing gene-environment interactions. He noted that the results for stratification by GSTT1 status lent credibility to the relevance of the glutathione pathway; however, more-critical evaluation of the evidence was needed. Two studies, Brüning *et al.* (2003) and Moore *et al.* (2010), considered GST polymorphisms. The odds ratios from Moore *et al.* were included in the table, and a test for interaction probably should also be included. It should be noted that studies are generally underpowered for assessing gene-environment interactions, and that this would be particularly true of the very small study by Brüning *et al*. The forest plot based on broad rank exposure categories may be misleading because the high exposure category includes two studies of low quality. He questioned whether the plot should be included and noted the text was more critical. In response to the public comments, he noted that the notion that relative risk must be greater than 2 to demonstrate a causal association was outdated; relative risks as low as 1.25 are used as sufficient evidence for carcinogenicity in, for example, IARC evaluations.

Dr. Silver raised the study-quality issues of recall bias and the use of proxy respondents, which could be an issue less for trichloroethylene exposure than for covariates such as smoking. Dr. Lash objected to the monograph's references to "non-

significant increases." If a result is not significantly significant, it cannot be called an "increase." Dr. Richardson asked why Brüning *et al.* (2003) was singled out for "other concerns" in Figure 4-1. Dr. Lunn said exposure was assessed at a later time for the controls than for the cases.

V.A.3.4 Presentation on Mechanism Studies

Dr. Sanford Garner, ILS, presented an overview of the key information in the draft monograph section on mechanistic data for kidney carcinogenicity. The NTP concluded that there is credible mechanistic evidence for renal carcinogenicity of trichloroethylene based on (1) tissue-site concordance for kidney cancer in humans and rats and (2) toxicokinetic and mechanistic data in both humans and animals providing evidence for biologically plausible modes of action for trichloroethylene's carcinogenicity in humans. The key events are (1) *in situ* production or systemic distribution to the kidneys of glutathione-conjugation-derived metabolites, (2) mutagenic and genotoxic effects induced by GST-mediated metabolites, and (3) cytotoxicity (nephrotoxicity) and regenerative cellular proliferation.

In studies in experimental animals, trichloroethylene caused kidney tumors in male rats exposed by inhalation or stomach tube. Humans and rodents metabolize trichloroethylene by both oxidative and glutathione-conjugation pathways and have similar mixtures of trichloroethylene metabolites in their tissues. Among the glutathione-conjugation-derived metabolites, DCVG has been in found in the blood of humans and rodents, and NAcDCVC has been found in the urine of humans and rodents. An important finding related to a potential mechanism for the carcinogenicity of glutathione-conjugation-derived metabolites in humans is that renal-cell cancer was significantly associated with exposure to trichloroethylene in individuals with at least one intact GSTT1 allele but not in GSTT1-null individuals (Moore *et al.,* 2010). In rodent kidney cells *in vivo,* oral exposure of rats to trichloroethylene increased micronucleus formation, and oral exposure of rats and mice to DCVC increased DNA strand breaks. DCVC also induced gene mutation, unscheduled DNA synthesis, and cell transformation in various other cell types. In humans, inactivation of the *VHL* tumor suppressor gene is thought to be an early and causative event in renal clear-cell carcinoma; however, epidemiological studies of *VHL* mutations and exposure to trichloroethylene were inconclusive.

Exposure to trichloroethylene is associated with nephrotoxicity in humans. DCVC causes necrosis in human proximal tubule cells *in vitro* at high concentrations and increased cell proliferation and apoptosis at lower concentrations and is nephrotoxic in rodents and other species. Rats and mice exposed to DCVC in drinking water showed nephrotoxicity progressing from tubular necrosis to increased karyomegaly and cytomegaly, effects similar to those seen with chronic exposure to trichloroethylene. Although cytotoxicity alone is insufficient for tumor formation, chronic tubular damage has been proposed as a precondition for nephrocarcinogenic effects of trichloroethylene in humans. Hypothesized modes of action have also been proposed for oxidative metabolites of trichloroethylene, including peroxisome proliferation activated receptor α (PPARα) activation, α_{2u}-globulin-related nephropathy, and formic acid-related nephrotoxicity, but the evidence for these mechanisms is weak.

Dr. Cullen asked how the difference in injury and tumor risk between the kidney and liver was explained, given that the glutathione conjugation pathway is active in both kidney and liver. Dr. Lash noted that the difference is primarily in the way the glutathione-conjugated metabolites are handled by the organs; the liver is very efficient at eliminating the metabolites in the bile.

V.A.3.5 Peer-Review Comments on Mechanism Studies

Dr. Lash, first reviewer, found the draft monograph section to be clear, concise, and well organized, and he particularly liked the table summarizing the proposed mechanisms. However, the relevance of the proposed mechanisms to human cancer generally needed to be made clearer. For example, the way in which the proposed formic acid mechanism was presented gave too much credence to this hypothesis, though the appropriate conclusion was reached. Dr. Lash noted that the written comments from the HSIA inaccurately stated that DCVC was not a highly potent kidney toxicant. Dr. Lash commented that in potential modes of action for renal cancer, there is a balance between cytotoxicity and changes that can lead to transformation, and that exposures leading to cytotoxicity and regenerative proliferation have been considered more important than genotoxic modes of action in the mechanism of carcinogenesis.

Dr. Douglas, second reviewer, said the data on mutagenicity of trichloroethylene metabolites in the kidney were limited; there was some evidence for *in vitro* effects, but not strong, convincing evidence for *in vivo* effects. He disagreed with the statements that there was strong evidence for a mutagenic mode of action; he would call the evidence “presumptive.” He agreed with Dr. Lash on the possibility of cytotoxicity-induced gene mutation and reiterated that for mutations to form from DNA adducts, there must be tissue turnover. Dr. Douglas suggested addressing the issue of whether the metabolite dose levels causing mutagenic effects *in vitro* could occur *in vivo*. He also found the paragraph discussing Douglas *et al.* (1999) confusing and thought that a simpler hypothesis could be proposed in interpretation of the results.

V.A.3.6 Panel Discussion of Mechanism Studies

Dr. Eastmond agreed somewhat with Dr. Douglas that while there was some evidence for *in vivo* genotoxicity of trichloroethylene, the conclusions about genotoxicity, as a mechanism of carcinogenicity, should be toned down. The modest numbers of tumors and extensive toxicity seen in experimental animals exposed to trichloroethylene, even at lower doses, might suggest a more important mechanistic role for cytotoxicity, likely in conjunction with genotoxicity. In the last sentence of the section, he suggested changing “the data are sufficient to conclude” to “the data support the conclusion,” noting that the conclusion was informed by a combination of evidence, including human epidemiological data. Dr. Douglas felt that “support” was too strong a word. Dr. Lash suggested saying that the data were “consistent with some role” for a mutagenic mode of action.

V.A.3.7 Presentation on Integration of the Kidney Cancer Data

In summary, Dr. Lunn stated that epidemiological studies have demonstrated a causal association between exposure to trichloroethylene and kidney cancer that cannot be

explained by chance, bias, or confounding. The evidence across studies was consistent, and high-quality studies showed evidence of an exposure-response relationship. Exposure to trichloroethylene causes kidney cancer in male rats. Toxicological and mechanistic data provide credible evidence for the biological plausibility of a proposed mutagenic and cytogenetic mode of action mediated by glutathione-conjugated metabolites, the key events of which likely occur in humans.

The NTP's preliminary level of evidence conclusion is that there is *sufficient evidence of a causal relationship between exposure to trichloroethylene and kidney cancer.*
Dr. Douglas asked whether the determination of carcinogenicity depended on the cancer data per se or on the combination of the cancer data with the mechanistic data — specifically, whether the mode of action had to be understood in order to conclude that there was sufficient evidence for carcinogenicity. Dr. Bucher advised that the vote should be based on each panelist's evaluation of the data and that mechanistic evidence is not required for a conclusion of sufficient evidence of carcinogenicity in humans. He said that changes of wording in the conclusion could be suggested in discussion of the preliminary listing recommendation.

V.A.3.8 Action

Dr. Richardson moved to accept the preliminary level of evidence conclusion, and Dr. Hammond seconded the motion. 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 tricholorethylene and kidney cancer. Dr. Douglas abstained because although he agreed there was sufficient evidence for carcinogenicity from the epidemiological studies, he did not consider that the mechanistic data contributed to the sufficient evidence.

V.A.4 Non-Hodgkin Lymphoma

V.A.4.1 Presentation on Human Cancer Studies

Dr. Ratcliffe, ILS, presented an overview of the key information in the draft monograph section on human studies of NHL and related lymphoma subtypes. The main, potentially confounding co-exposures are to benzene, phenoxy herbicides, ionizing radiation, and chlorinated or other organic solvents. Ten cohort or nested case-control studies, seven case-control studies, and two meta-analyses were reviewed. Most of the studies were of low or low/moderate quality and had limited sensitivity to detect an association. Two of the low quality studies had methodological concerns, and the lowest-ranked study had potential for bias that would likely lead to an overestimation of the risk estimate. The most informative studies were a large pooled European case-control study (Cocco *et al.,* 2013), considered to be of high quality, a pooled Nordic cohort study, and a cohort mortality study of U.S. aircraft workers, both considered to be of moderate quality.

The NTP concluded that there is limited evidence of an association between increased risk of NHL or related subtypes and exposure to trichloroethylene based on (1)

moderately elevated risks observed in several studies with different study designs and in different populations (though the strength of evidence varied across studies), (2) a relatively strong association and positive exposure-response trends in the most informative study (Cocco *et al.,* 2013) and one of its component studies (Purdue *et al.,* 2011), and (3) the suggestion of statistically significant increased risk for NHL across studies in the meta-analyses. Limitations of the evidence include (1) the lack of strong association and exposure-response relationships in the cohort studies, (2) methodological limitations of some case-control studies, and (3) potential confounding by co-exposure to chlorinated organic solvents in some studies (e.g., of aircraft workers).

A forest plot of relative risk estimates for ever-exposed individuals in each study, stratified by study quality, showed modestly increased risks in several studies. The highest relative risk was from the study with a potential positive bias; this study did not adjust for co-exposure to phenoxy herbicides. The large pooled case-control study (Cocco *et al.,* 2013) used several exposure metrics and had the statistical power to look at subtypes. Using the Fisher's test for combined probability (probability, duration, frequency, and intensity of exposure), significant associations were found for NHL, follicular cell lymphoma (FCL) and chronic lymphocytic leukemia (CLL). Positive exposure-response associations with exposure level or duration were found for NHL, FCL, or CLL among subjects with a high probability of exposure in the pooled analysis and in one of, the component studies. (Purdue *et al.,* 2011). Both meta-analyses found significantly increased risks for ever-exposure. One of the meta-analyses also found a significantly increased risk for the highest exposure groups, with low sensitivity to removal of studies or selection of alternative relative risks, low to moderate heterogeneity, and some evidence of publication bias.

In response to questions from Dr. Cantor, Dr. Ratcliffe noted that because other chlorinated solvents may be a risk factor for NHL, the fact that some of the relevant occupational studies did not adjust for these co-exposures was a limitation for evaluation of NHL, but not kidney cancer. She also clarified that Cocco *et al.* re-evaluated the exposure data in the pooled studies, so she could not say what the effect would be of removing the Purdue *et al.* data from the pooled analysis. Dr. Lunn said Cocco *et al.* reported that there was no heterogeneity among the pooled studies.

V.A.4.2 Peer Review Comments on Human Cancer Studies

Dr. Vineis, first reviewer, found the review to be quite accurate. It is very useful to have information on the classifications used for NHL, as these have changed over time, possibly introducing some degree of misclassification, particularly in the updates of the cohort studies. As with the kidney cancer studies, he would like to see a more explicit link between evaluation of the individual studies and the quality ranking. It also would be useful to have information about response rates in the tables. He noted that the high quality of Cocco *et al.* was related to the good exposure assessment, but the component studies had limitations (primarily response rates), and the evaluation for potential bias in each of the component studies should be more explicit.

Dr. Cantor, second reviewer, agreed with the integration of the information, but suggested that more detailed information should be provided on secular trends in NHL. He thought there was less concern about confounding in these sets of studies, as the risk factors are not common in the general population. He agreed that information on the response rates in the component studies of the pooled analysis should be clearly presented.

Dr. Richardson, third reviewer, said it would be useful to clarify the implications of changing classifications of NHL. The distribution of NHL subtypes varies geographically and over time, which may lead to heterogeneity in results if only some subtypes are caused by trichloroethylene. Also, the cohort mortality studies likely did not include CLL in the category of NHL, whereas CLL may account for about 20% to 25% of total NHL cases in the recent incidence studies. Dr. Richardson noted that the interpretation of Hardell *et al.* (1994) did not seem to follow the study evaluation framework described in Section 3, as inconsistency with the results of other studies was given as a reason for suspecting bias or confounding.

V.A.4.3 Presentation on Mechanism Studies

Dr. Andrew Ewens, ILS, presented an overview of the key information in the draft monograph section on mechanistic data for NHL. Trichloroethylene exposure by inhalation is associated with lymphomas in humans and in female mice. Little is known about the mechanisms of NHL; however, the vast majority of these lymphomas originate from B cells, and immunomodulation is a strong risk factor for B-cell NHL. Immune biomarkers for trichloroethylene exposure have been measured in humans and animals, although no study has directly evaluated immunomodulation as a mode of action for trichloroethylene-associated NHL.

Dr. Ewens stated that immunomodulation (both immunosuppression and autoimmunity) is linked to cancer, including NHL. Organ-transplant patients, HIV patients, and genetically immunodeficient patients are at increased risk for NHL. The incidence of NHL is also increased in individuals with autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, and scleroderma.

Dr. Ewens described a proposed model (Ponce *et al.,* 2014) of NHL induction via antigen-induced B-cell activation. B cells are the only immune cells that undergo somatic mutation after initial maturation. The progenitor B cell undergoes DNA recombination to generate the antigen-specific portion of the antibody. Activation of the B cell by the antigen initiates a second round of DNA recombination, and antigen specificity is refined by point mutations. The mutations change the antigen-binding region, and the B-cells that bind more strongly to the antigen are more likely to be reactivated on continual exposure to the antigen; thus, the risk of damaging mutations increases as the antigen persists.

In epidemiological studies, trichloroethylene exposure was associated with increased incidence of scleroderma and increased production of antibodies in patients with hypersensitive skin disorders; decreased peripheral blood lymphocytes, B-cell activation, and antibodies; and reactivation of herpes virus; the results for changes in cytokines were inconclusive. In experimental animals (primarily transgenic autoimmune-

prone mice), trichloroethylene exposure resulted in increased protein adducts, anti-adduct and anti-self antibodies, and autoimmune hepatitis, presumably as a result of B-cell activation. Also observed were decreased peripheral leukocytes and bacteria-fighting immune cells, resulting in increased mortality and persistent infections. Limitations of the mechanistic data included the small number of studies on trichloroethylene-induced immunomodulation in humans, the use of a transgenic mouse model in most animal studies, the use of different endpoints in animal and human studies, the lack of studies on immunomodulation and cancer, and inconsistencies of some B-cell-activity results with the proposed model. The NTP concluded that the available studies do not provide convincing evidence that trichloroethylene causes NHL via the proposed immunomodulatory mode of action, but that trichloroethylene-induced immunomodulation resulting in NHL is biologically plausible.

Dr. Sarah Blossom noted that the draft monograph emphasized immunosuppression as a potential mechanism of action and asked whether the NTP was moving away from that interpretation and towards immunomodulation. Dr. Ewens noted that not all of the data are consistent with immunosuppression.

V.A.4.4 Peer Review Comments on Mechanism Studies

Dr. Vineis, first reviewer, stressed the importance of considering the data on immunosuppression and autoimmunity in NHL outside the context of the proposed model. He found it impressive that the same phenomena have been associated with the etiology of NHL and with trichloroethylene exposure. However, he stated that the review of the epidemiologic studies on immune effects was less rigorous than that of the review of the human cancer studies as less information about the studies was provided, the quality of the studies was not clear, and publication bias was not addressed. The quality of the epidemiological review of the immune studies would be improved by more detail, especially on scleroderma and autoimmune diseases and immune endpoints in the Chinese studies.

Dr. Blossom, second reviewer, said much of the evidence presented on immunosuppression in humans was fairly weak, because the studies that were considered most informative relied on phenotypic evaluation of circulating lymphocytes, and a decline in the numbers of peripheral blood lymphocytes does not indicate functional immune suppression. Because the Chinese workers in those studies developed a severe hypersensitivity skin reaction and immune-mediated liver inflammation, those cells could be infiltrating other tissues. She thought that the Chinese studies indicated immune hyperactivity, not immune suppression, and that data on immune suppression in humans exposed to trichloroethylene were very limited. The disparity of responses seen in animal studies could be related to differences in species, strain, mode of exposure, and dose level, which were not clearly defined in the draft monograph. She also noted that in both studies showing the inability of mice to fight off infection, phagocytic activity was actually increased at certain doses of trichloroethylene. Although trichloroethylene is clearly immunotoxic, it is a stretch to conclude that it is immunosuppressive.

Dr. Ewens said the approach taken in the draft monograph was to review endpoints consistent with immunosuppression, which was not necessarily sufficient to indicate it. He noted that the evidence for a role of extravasation in decreased peripheral-blood leukocytes or lymphocytes was inconsistent in animal studies, possibly related to the timing of the observations. He added that because the antibacterial response is a complex, multi-step process, inconsistent results in the studies of bacterially challenged mice could have been related to the timing of the observations.

V.A.4.5 Panel Discussion of Mechanism Studies

Dr. Cantor mentioned a paper in press by Morton *et al.* that addresses heterogeneity among NHL subtypes. Although it does not specifically analyze trichloroethylene exposure, it could be added to the general introductory material. Dr. Eastmond commented that lymphomas induced in mice are often T-cell lymphomas, which may not be of the same origin as the B-cell lymphomas.

V.A.4.6 Presentation on Integration of the NHL Data

In summary, Dr. Lunn stated that epidemiology studies provided limited evidence of an association between exposure to trichloroethylene and NHL in humans. The conclusion of NTP's previous evaluation was that trichloroethylene causes lymphoma in experimental animals. The toxicological and mechanistic evidence for trichloroethylene-induced immunomodulation leading to NHL is biologically plausible, but not conclusive. The NTP's preliminary level-of-evidence conclusion is that there is *limited evidence of a causal association between exposure to trichloroethylene and NHL from studies in humans.*

V.A.4.7 Action

Dr. Cantor moved to accept the preliminary level of evidence conclusion, and Dr. Cullen seconded the motion. 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.*

V.A.5 Liver Cancer

V.A.5.1 Presentation

Dr. Garner presented an overview of the key information in the draft monograph section on liver cancer. Liver cancer is relatively rare and has a relatively low survival rate. The main potentially confounding co-exposures are to vinyl chloride, X- and gamma radiation, alcohol consumption, and tobacco smoking. Twelve cohort or nested case-control studies were reviewed, as well as one case-control study that included only one case among exposed individuals, and two meta-analyses. Some studies reported only on primary liver cancer, others on combined cancer of the liver and intrahepatic bile ducts, and a few on combined cancer of the liver, intrahepatic and extrahepatic bile ducts, and gallbladder. Study quality was low to moderate, the major limitation being limited sensitivity to evaluate risks or exposure-response relationships. One of the low-

ranked studies, of uranium-processing workers, was considered to have potential confounding from exposure to ionizing radiation.

The NTP concluded that the epidemiological data are inadequate to evaluate the relationship between liver cancer and exposure to trichloroethylene because (1) evidence for an association came primarily from a few cohort studies and statistically significant increased risks in the two meta-analyses, (2) evidence from studies published since the 2011 meta-analysis appears to be weaker, (3) there is little evidence for an exposure-response relationship, (4) confounding cannot be ruled out, especially in the aircraft manufacturing studies, where exposures to other halogenated solvents also caused liver cancer in rodents, and (5) the findings are inconsistent across studies.

In experimental animals, trichloroethylene exposure by inhalation or stomach tube caused liver tumors in mice of both sexes. The available data support a role for oxidative metabolites of trichloroethylene in liver carcinogenicity, but suggest that the mode of action is complex and likely involves key events from several pathways. The liver in both humans and animals is exposed to a similar mixture of oxidative metabolites. However, chloral hydrate concentrations *in vivo* appear to be less than those that cause genotoxicity *in vitro,* and neither TCA nor DCA alone causes the full spectrum of characteristics of trichloroethylene-induced liver tumors. There is some supporting evidence for several biologically plausible modes of action, including mechanisms potentially relevant to humans. Proposed modes of action include genotoxicity, PPARα activation, oxidative stress, epigenetic changes, and autoimmune hepatitis, but no one mechanism has adequate supporting data, interactions between several modes of action are possible, and key events from several pathways may be involved.

V.A.5.2 Peer Review Comments

Dr. Parent, first reviewer, said that the scientific information on human cancer studies was clear, technically correct, and objectively presented. The studies' power to detect an effect was a significant issue, and the relative risks were likely to have been sensitive to other methodological shortcomings. The two meta-analyses provided the most positive information, but the monograph should further discuss their limitations and give them less weight. Although they both found significant meta relative risks, the authors of both meta-analyses concluded that the data on liver cancer were too limited. Dr. Parent mentioned a paper by Turner *et al.* (2013) that assessed the contributions of small, underpowered studies to meta-analyses, finding them to be limited.

Dr. Vineis, second reviewer, agreed that more discussion of the meta-analyses was needed to clarify why the evidence for liver cancer was inadequate. He suggested that Table 6-2 understated the heterogeneity of the studies. In response, Dr. Lunn noted that the forest plot of studies reviewed in the draft RoC monograph showed more heterogeneity than the meta-analyses because it included more recent studies than the published meta-analyses.

Dr. Cullen, third reviewer, said that overall, the section on mechanisms of liver cancer was clear and technically correct, and the data were objectively presented. The

overarching conclusion that the mode of action is likely complex and that the data are currently inadequate to generate a definite mode of action was appropriate. However, the conclusion of the section on PPARα activation was too strong, and the section on epigenetic changes addressed issues that might not be relevant to liver cancer, including the discussions of effects on macrophage activation and SET-associated proteins. Dr. Cullen also questioned the relevance of the section on autoimmune hepatitis and the autoimmune-prone mouse model, as autoimmune hepatitis is not a risk factor for primary hepatocellular carcinoma in humans.

Dr. Lash, fourth reviewer, said the information was well organized and clearly summarized, and that evaluation of the relative importance of the proposed mechanisms was needed. Although he agreed with the conclusion that the data were insufficient for a firm conclusion about the mechanism, he noted that the evidence and relevance to humans is weaker for some mechanisms, such as PPARα activation.

Dr. Blossom, fifth reviewer, found the section to be well written, technically correct, and thorough. She concurred with the comments of Drs. Cullen and Lash.

V.A.5.3 Panel Discussion

Dr. Douglas noted that in Section 2.7.2, the source of the statement concerning induction of *lacI* mutations in transgenic mice by DCA (Leavitt *et al.* 1997) was not cited or included in the reference list. These mutations were induced in the liver, and this study should be cited in Section 6. Dr. Eastmond noted that he had participated in another review of the studies on H-*ras* mutation frequency and spectrum of tumors induced by TCA in mouse liver, in which it was suggested that TCA was enhancing spontaneous tumors.

V.A.5.4 Presentation on Integration of the Liver Cancer Data

In summary, Dr. Lunn stated that the data available from studies in humans are inadequate to evaluate the relationship between liver cancer and exposure to trichloroethylene, because of inconsistent findings from studies in humans with little evidence for exposure-response relationships and limited ability of the human studies to evaluate rare cancer such as liver cancer. Mechanism(s) of liver carcinogenicity have not been established but are likely complex, involving key events from multiple modes of action. No data suggest that trichloroethylene causes liver tumors in mice by mechanisms that are not relevant to humans.

The NTP's preliminary level of evidence conclusion is that the studies in humans are *inadequate* to evaluate the relationship between liver cancer and exposure to trichloroethylene.

V.A.5.5 Action

Dr. Parent moved to accept the preliminary level of evidence conclusion, and Dr. Cullen seconded the motion. 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 there is *inadequate evidence of a causal relationship between exposure to trichloroethylene and liver cancer.* This conclusion is

based on human epidemiological studies, together with toxicokinetic, toxicological, and mechanistic studies.

V.A.6 Overall Cancer Evaluation

Dr. Lunn presented an overview of the overall cancer evaluation in the draft monograph. The preliminary listing recommendation was that trichloroethylene is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans. Human epidemiological studies together with toxicokinetic, toxicological, and mechanistic studies show that trichloroethylene causes kidney cancer in humans. There is limited evidence for the carcinogenicity of trichloroethylene from studies of NHL in humans. Supporting evidence is provided by studies in experimental animals.

In discussion by the panel, the wording of the preliminary listing recommendation was slightly revised (as shown below, under Action).

V.A.6.1 Action

Dr. Cullen moved to accept the preliminary listing recommendation as revised, and Dr. Lash seconded the motion. The Panel agreed unanimously (9 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision 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. 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 NHL from studies in humans. Supporting evidence is provided by studies in experimental animals, which demonstrate that trichloroethylene causes tumors at several tissue sites.

V.B. Draft RoC Substance Profile

Mr. Alton Peters, ILS, summarized the updated environmental exposure information in the draft substance profile, which includes the latest information from the EPA's Toxics Release Inventory (TRI) database. From 1988 through 2011, environmental releases of trichloroethylene declined over 95%. Based on data from the National Health and Nutrition Examination Survey (NHANES) from 1988 through 2006, trichloroethylene blood levels are decreasing in the general population. The percent of the U.S. population with detectable blood levels of trichloroethylene declined from 10-12% for 1988 through 2000 to below the limit of detection (LOD) for 2001 through 2006. However, cases of trichloroethylene exposure have been reported recently for certain populations, including those near Superfund sites in Asheville, NC, and Mountain View, CA.

Dr. Hammond noted that the draft substance profile reports that NHANES trichloroethylene blood levels in 2005–2006 were below the LOD for the 95% percentile of the population, which would imply that no more than 5% of the population had blood levels of trichloroethylene above the LOD of 0.012 ng/mL.

V.B.1.1 Peer Review Comments and Panel Discussion

Dr. Lash, first reviewer, thought the profile provided a clear and succinct summary of the information.

Dr. Vineis, second reviewer, suggested adding a discussion of inequalities of exposure across populations.

Dr. Hammond, third reviewer, emphasized that the decline in trichloroethylene exposure of the general population and the exposure in specific areas with remaining high trichloroethylene levels are independent points. She noted that the nature of the available literature on exposure, coming from different time periods, tended to misrepresent current exposure and disguise trends. For example, taken together, the U.S. export data indicated an 85% decrease from 1992 to 2013, consistent with the 95% decline in TRI emissions. Some of the information on use (e.g., as a metal degreaser) and sources of exposure (e.g., in consumer products) appeared to be outdated. It would also be worth looking at trends in numbers of exposed workers and in their exposure levels. Dr. Hammond suggested that the sections on exposure be rewritten, possibly organized by exposure source and time period, to acknowledge changes in use and exposure and limitations in the available data. Dr. Garner noted that after the draft substance profile was written, a new EPA publication was found with good information on changing use of trichloroethylene. He also noted that the information on exposure in consumer products was up to date.

Dr. Eastmond suggested that the section on liver cancer should emphasize that some of the evidence came from experimental animals. He also suggested mentioning the potential for dermal exposure to trichloroethylene in water during showering. Dr. Douglas considered the statement, “the available mechanistic data strongly support a mutagenic mode of action [for kidney cancer] mediated by glutathione-conjugated metabolites,” to be too strong.

VI. Closing Remarks on Draft RoC Monograph

Dr. Bucher thanked the panel for their thorough reviews and helpful comments.

The meeting was adjourned at 4:40 p.m.

VII. References Cited

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