National Toxicology Program

Peer Review of Draft Report on Carcinogens (RoC) Monographs on *ortho*-Toluidine and Pentachlorophenol and By-products of Its Synthesis

December 12–13, 2013

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

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Draft Peer Review Report

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I. Attendees

Peer Review Panel
Kenneth McMartin (Chair), Louisiana State University
Stelvio Bandiera, University of British Columbia
Laura Beane Freeman, National Cancer Institute
Stephen Nesnow, Independent Consultant
Gabriele Sabbioni, Tulane University (reviewed *ortho*-toluidine monograph only)
Martha Sandy, California Environmental Protection Agency
MaryJane Selgrade, ICF International
Allan Smith, University of California, Berkeley
Glenn Talaska, University of Cincinnati
Paul Villeneuve, Carleton University, Ottawa, Canada
Elizabeth Ward, American Cancer Society
Shelia Zahm, Independent Consultant

National Toxicology Program Board of Scientific Counselors Liaison
Lisa Peterson, University of Minnesota

Other Federal Agency Staff
Tania Carreón-Valencia, National Institute for Occupational Safety and Health (NIOSH, by telephone)
Glinda Cooper, Environmental Protection Agency (EPA, by telephone)
Gayle DeBord, NIOSH (December 12 only)
Kevin Dunn, NIOSH (by telephone)
Tim McMahon, EPA (by telephone)
Avima Ruder, NIOSH (by telephone)
Mary Schubauer-Berigan, NIOSH (by telephone)

National Institute of Environmental Health Sciences Staff
John Bucher
Gloria Jahnke
Grace Kissling
Kelly Lenox
Ruth Lunn
Robin Mackar
David Malarkey
Diane Spencer
Nigel Walker
Lori White
Mary Wolfe
Yun Xie

Report on Carcinogens Contract Support Staff
Stanley Atwood, Integrated Laboratory Systems (ILS)
Ella Darden, ILS
Susan Dakin, Independent Consultant
Sanford Garner, ILS
Alton Peters, ILS
Jennifer Ratcliffe, ILS
Carol Swartz, ILS

Public Attendees
Ernie Hood, Bridport Services
Lauren Stranahan, North Carolina State University
Robert Golden, ToxLogic LC (by telephone)

II. Welcome and Introductions

The National Toxicology Program (NTP) Peer Review Panel for the Draft Report on Carcinogens (RoC) Monographs for *ortho*-Toluidine and Pentachlorophenol and By-products of Its Synthesis convened on December 12 in Rodbell Auditorium, Rall Building, National Institute for Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Kenneth McMartin served as chair. Dr. Lisa Peterson attended as the NTP Board of Scientific Counselors (BSC) liaison. Cdr. Gayle DeBord attended representing the National Institute for Occupational Safety and Health (NIOSH) (December 12 only). Representing the NTP were NTP Associate Director Dr. John Bucher; Dr. Mary Wolfe, Deputy Division Director for Policy; Dr. Ruth Lunn, Director, Office of the RoC; and Dr. Gloria Jahnke, Health Scientist, Office of the RoC. Dr. Lori White, Staff Scientist, Office of Liaison, Policy and Review, served as the Designated Federal Official.

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

III. Process for Preparing the Draft RoC Monographs

III.A. Presentation

Dr. Lunn presented background information on the RoC and on the process and methods used to prepare the draft RoC monographs for *ortho*-toluidine and pentachlorophenol and by-products of its synthesis. She noted that for every candidate 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, the draft RoC monograph is written, which consists of two parts: (1) a literature-based cancer evaluation and (2) the draft substance profile, comprised of the preliminary listing recommendation and a summary of the scientific evidence considered to be key for reaching the recommendation. The RoC is a cumulative compilation of the profiles for all listed substances.

The process for preparing the RoC (revised in 2012 and used for the first time in preparation of the 13th RoC) consists of the following steps: (1) nomination and selection of the candidate substances, (2) scientific evaluation in draft monographs, (3) public release and peer review of the draft monographs, and (4) submission of the substance profiles to the Secretary of Health and Human Services (HHS) for approval. The process provides opportunities for public comment, scientific input, and peer review of the scientific information.

Dr. Lunn outlined the specific steps of the review process that had been completed for *o*-toluidine and pentachlorophenol. In January 2012, a **Federal Register** notice was published inviting public comment on 20 nominations, including *o*-toluidine and pentachlorophenol. Public comments were received on these two substances. In June 2012, the draft concept documents were reviewed by the BSC in a public meeting, and both *o*-toluidine and pentachlorophenol were selected as candidate substances.

Dr. Lunn noted that the cancer evaluation process allows flexibility in how scientific and public inputs are obtained, depending on the complexity of the substances. For *o*-toluidine, technical advisors were identified who had expertise in dyes, epidemiology, and absorption, distribution, metabolism, and excretion (ADME). Materials posted on the RoC website included an expert report on the dyes found in the epidemiologic studies of occupational exposure to *o*-toluidine, the protocol for evaluating human cancer studies, and the literature search strategy and list of references. After the cancer evaluation was completed, the substance profile was drafted and underwent interagency review, and the draft monograph was completed in August 2013. The process for pentachlorophenol was similar. Additional steps were taken to address the complexity of pentachlorophenol's dioxin by-products: a public webinar was conducted to address by-products in human studies, and an informational group was convened to address by-products in animal studies.

Dr. Lunn outlined the structure of the draft monographs, noting that they are not intended to be encyclopedic, but rather to focus on the issues relevant to evaluating carcinogenicity. She also reviewed the methods used to prepare the draft monograph and the criteria used to assess the literature in each discipline. Steps followed in preparation of the cancer evaluation component are: (1) identify scientific issues and develop key questions, (2) identify and select literature using a systematic literature search, (3) extract data and describe studies, (4) assess the quality of studies, (5) synthesize the findings across studies and reach level of evidence conclusions for each discipline, and (6) integrate the overall body of evidence and reach a preliminary RoC listing recommendation.

With respect to exposure, she noted that the Public Health Service Act requires that the RoC list substances "to which a significant number of persons residing in the United States are exposed." Because this information rarely is available, it typically has been inferred from data on use, production volume, occupational monitoring, environmental occurrence, estimated daily intake, biomonitoring of the general public, and past exposure. She asked the peer reviewers to use their judgment in deciding whether the exposure data in the draft monographs supported the conclusion that a significant number of U.S. residents are exposed to these substances.

The preliminary listing recommendation is based on applying the RoC criteria to the data on cancer in humans, cancer in experimental animals, and mechanisms of carcinogenicity. These criteria are used to make decisions about the level of evidence for cancer in humans and in animals and to reach conclusions about potential mechanisms of action. This body of knowledge is integrated to form the basis for the listing recommendation.

Dr. Lunn 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 sufficient evidence of carcinogenicity from studies in experimental animals. She then reviewed

the RoC criteria for listing of substances as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen.* She emphasized that conclusions regarding carcinogenicity in humans or experimental animals are based on consideration of all relevant scientific information, as outlined in the RoC listing criteria.

The charge to the Peer Review Panel was as follows:

(1) 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.

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

For each substance, the Panel would be asked to vote on the following questions:

(1) Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from cancer studies in humans.

(2) Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from cancer studies in experimental animals.

(3) Whether the scientific evidence supports the NTP's preliminary policy decision on the RoC listing status of the substance.

Following the peer review, the draft monographs are revised based on the peer-review comments, information about the peer review and NTP's response to the Panel's listing recommendations is provided to the BSC, after which the monographs are finalized. Once all reviews have been completed for the next edition of the RoC, the substance profiles for newly reviewed candidate substances are submitted to the Secretary, HHS, for approval or disapproval and the next edition of the RoC is published.

IV. Draft RoC Monograph for *ortho*-Toluidine

IV.A. Oral Public Comments

There were no oral public comments on the Draft RoC Monograph for *ortho*-Toluidine.

IV.B. Written Public Comments

Dr. Wolfe said the NTP had received one written public comment, from Steven Wodka, Attorney at Law. This comment was provided to the Panel to be carefully considered in their review and was posted to the NTP Web site. The following major scientific issues raised in the public comment were identified:

- Mr. Wodka endorsed listing of *o*-toluidine as *known to be a human carcinogen.*
- He disagreed with the statements in Freeman report (Freeman, 2012) that current standards pertaining to ventilation and lab coats and gloves protect workers against exposures and that the use of *o*-toluidine in dye manufacturing has been largely banned.

- He asked that the NTP clarify that the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) and American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value–time-weighted average (TLV-TWA) cited in the substance profile are based on toxic effects other than cancer.

IV.C. Draft Cancer Evaluation Component

IV.C.1 Properties and Human Exposure

IV.C.1.1 Presentation

Dr. Lunn presented an overview of the key information in the draft monograph. *o*-Toluidine is an aromatic amine used to make dyes, rubber chemicals, and herbicides. It has been listed in the RoC since 1983 as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals. The NTP decided to re-review *o*-toluidine because numerous human cancer studies have been published since 1983 and there is widespread U.S. exposure to *o*-toluidine.

The evidence suggests that a significant number of U.S. residents are (or were) exposed to *o*-toluidine. Although the pattern of use of *o*-toluidine has changed over time, ongoing uses include as dye intermediates and herbicide precursors and in the manufacture of rubber chemicals and prilocaine. In addition, exposure occurs during the production of *o*-toluidine. The primary routes of occupational exposure are inhalation and dermal contact. Average workplace levels have typically been 0.1 ppm or less, decreasing over time. Evidence for exposure outside the workplace includes detection of *o*-toluidine in urine and breast milk, *o*-toluidine–hemoglobin (Hb) adducts in the blood, and *o*-toluidine–releasing DNA adducts in urinary bladder tissue or tumors. Potential sources of non-occupational exposure to *o*-toluidine include cigarette smoke, use of prilocaine as a dental anesthetic, use of hair dyes and other dyes, its presence in food (based on European food surveys), and its presence in the environment (based on U.S. EPA Toxics Release Inventory [TRI] data).

IV.C.1.2 Peer Review Comments and Panel Discussion

Dr. Glenn Talaska, first reviewer, said the section on properties and human exposure was generally clear and well written. He suggested (1) adding the equilibrium concentrations of *o*-toluidine and its hydrochloride to Table 1-2; (2) adding the current PELs and TWAs to Section 1, particularly the skin notations for these substances; (3) adding information, if available, on whether prilocaine production and dental use are increasing or decreasing: and (4) having greater consistency in presenting units in the data. He suggested putting greater emphasis on: (1) the relative importance of dermal exposure to *o*-toluidine, especially in the workplace, given that the TLV is relatively high and air levels have decreased; (2) information on dermal absorption of *o*-toluidine; and (3) the importance of second-hand smoke from cigarettes, given that the levels of *o*-toluidine in second-hand smoke are 50 to 200 times the levels inhaled by smokers. Dr. Talaska said second-hand smoke could result in exposure of a significant portion of the population to *o*-toluidine.

Dr. Gabriele Sabbioni suggested using a structured search, such as Chemical Abstracts Service SciFinder, to identify products that could metabolically release *o*-toluidine. This search could provide a complete picture of exposure. In particular, exposure to *o*-toluidine via tattoo dyes should be considered. He suggested citing Skipper *et al.* (2010) on aromatic amines as potential human carcinogens. He said case-control studies found significant correlations between aromatic amines (other than *o*-toluidine) and urinary bladder cancer, possibly suggesting that other aromatic amines found in industrial settings are more dangerous than *o*-toluidine.

Dr. Sabbioni recommended that the work of Böhm *et al.* (2011) on urinary bladder DNA adducts should not be cited, because the acid hydrolysis method used to cleave the potential DNA adducts was not validated with synthetic DNA adducts. He said the standard procedure developed by other research groups uses base hydrolysis. Dr. Sabbioni noted that the monograph cites only the newest literature and relies on reviews rather than citing original papers, giving an unbalanced picture of the field.

Regarding the use of the acid hydrolysis method for cleaving DNA adducts, Dr. Stanley Atwood noted that the method had been validated for many other tobacco-specific nitrosamines. Dr. Sabbioni said the acid hydrolysis method had not been validated for *o*-toluidine.

Dr. Lunn acknowledged the importance of mentioning the skin notations in the regulatory exposure limits and guidelines for *o*-toluidine. She also noted that higher exposure to *o*-toluidine in second-hand smoke could have affected the results of studies comparing *o*-toluidine–Hb adducts between smokers and non-smokers in the general population. Dr. Talaska agreed, noting that occupational exposure to *o*-toluidine clearly dwarfs exposure from tobacco smoking. Dr. Allan Smith stressed the importance of documenting that current exposure standards are not based on cancer risks.

Dr. McMartin summarized the consensus of the Panel as in agreement that significant numbers of people in the United States are or were exposed to *o*-toluidine.

IV.C.2 Cancer Studies in Experimental Animals

IV.C.2.1 Presentation

Dr. Lunn presented an overview of the key information on studies in experimental animals. The current RoC listing of *o*-toluidine was based on several feeding studies in several strains of rats and mice; additional animal studies have been published since that time. All studies identified in the literature search were evaluated for reporting and quality for several performance elements The findings were consistent across the studies of adequate quality, and the results of the new studies supported those of the original studies.

The studies consistently showed that *o*-toluidine exposures causes urinary bladder tumors in rats, which are very rare in control animals. In a study of female rats, there was a high incidence of urinary bladder neoplasms, with a decreased time to first tumor and a dose response. In male rats, there were consistent findings of urinary bladder neoplasms in 3 chronic studies in 2 different strains. Also observed in rats were significantly increased incidences of tumors of connective tissue, subcutaneous tissue, mesothelium, and mammary gland.

The two chronic-exposure studies in mice, in both sexes of two different strains, were considered to be informative; one study used higher doses than the other. Blood-vessel tumors increased in both sexes in albino CD-1 mice (which was conducted at a higher dose) and in males in B6C3F1 mice. Liver tumors in female mice were considered to be treatment related in B63F1 mice. The studies of s.c. exposure to *o*-toluidine in hamsters and rats were considered to provide inadequate evidence of carcinogenicity due to limited exposure, small sample size, and uncertainty about relevance of exposure.

The NTP concluded that there is sufficient evidence of carcinogenicity in experimental animals based on increased incidences of tumors of the urinary bladder and connective tissue in rats of both sexes, subcutaneous tissue and mesothelium in male rats, blood vessels in mice of both sexes, and liver in female mice. Increased incidences of benign mammary gland tumors in rats of both sexes were considered to provide supporting evidence for the carcinogenicity of *o*-toluidine because they do not typically progress to malignant neoplasms.

Dr. Talaska commented that given the mechanism of activation of *o*-toluidine, liver tumors in rodents would be expected, because they are relatively fast acetylators; therefore, he found the dose-related occurrence of liver tumors in mice to be persuasive evidence, which should be emphasized in the monograph. Dr. Lunn said liver tumors were not emphasized in the discussion of mechanism because the focus was on the urinary bladder cancer observed in the human studies. Dr. MaryJane Selgrade asked why s.c. injection was not considered a relevant route of exposure, given the importance of dermal exposure in humans. Dr. Lunn said the focus was on metabolism of *o*-toluidine in the liver, followed by transport to the urinary bladder; she was not sure how much is known about dermal metabolism of *o*-toluidine.

IV.C.2.2 Peer Review Comments and Panel Discussion

Dr. Sandy, first reviewer, stated that the overall approach for preparing the cancer assessment of the experimental animal studies was appropriate, starting with the studies that were part of the basis for listing in 1983 and identifying additional studies published since that time. She found the scientific information to be clear, technically correct, and objectively presented. She suggested adding information on the possible progression of mammary gland fibroadenoma to adenocarcinoma in rats and considering whether to combine the two tumor types in the F344 male rat study. Dr. Malarkey said progression has occasionally been observed in female rats, and adenocarcinoma of the mammary gland is very rare in male rats. Dr. Sandy said the findings across the studies were synthesized appropriately, and she agreed with the preliminary conclusion of sufficient evidence of carcinogenicity of *o*-toluidine in experimental animals based on increased incidences of malignant tumors and combined malignant and benign tumors observed in multiple species and at multiple tissue sites in rats and mice, including the rare urinary bladder tumors in rats of both sexes.

IV.C.2.3 Action

Dr. Talaska moved that the Panel accept the preliminary conclusion that there is sufficient evidence of the carcinogenicity of *o*-toluidine from studies in experimental animals based on an increased incidence of tumors in two species and at several tissues sites: urinary bladder,

connective tissue, subcutaneous tissue, mesothelium, blood vessel, or liver. Dr. Sandy seconded the motion, and which passed unanimously (11 yes, 0 no, 0 abstentions).

IV.C.3 Cancer Studies in Humans

IV.C.3.1 Presentation

Dr. Lunn presented an overview of the key information in the draft monograph section on human cancer studies. Only urinary bladder cancer was evaluated, because the available data were not considered adequate for other tumor types. Based on the study-quality evaluation, the most informative studies were considered to be a 2010 NIOSH rubber chemical workers cohort study, a 2008 U.K. rubber chemical worker cohort study, and a 2010 Italian dye workers cohort study. Of these three studies, the NIOSH study was considered to be the most informative. Less informative studies were a 1954 U.K. dye workers cohort study (which did not specifically mention *o*-toluidine), a 1983 U.S. dye workers cohort study (which had a high potential for exposure misclassification), and a 2007 population-based case-control study (which had a high potential for exposure misclassification and did not adjust for other occupational exposures). A 1988 4-chloro-*o*-toluidine (4-COT) production worker cohort study was considered inadequate because of possible selection bias and because the main exposure was to 4-COT, for which there is limited evidence for urinary bladder carcinogenicity.

Dr. Lunn noted that an update of the 2010 NIOSH cohort study had recently been accepted for publication (Carreón *et al.* 2013); this paper was not cited in the draft monograph but was provided to the Panel for its review. This update adds 18 years of follow-up, improved case ascertainment, greater statistical power, improved exposure assessment, and more detailed statistical analyses. Because the updated study confirmed and strengthened the findings of the original study, the *o*-toluidine monograph will be revised to cite the 2013 study instead of the 2010 NIOSH cohort study. Like the 2010 study, the 2013 study found a significantly elevated risk of urinary bladder cancer among workers definitely exposed to *o*-toluidine, with a significant positive exposure duration–response relationship, and concluded that the elevated risk was unlikely to be due to tobacco smoking. In addition, the 2013 study found positive exposure-response relationships for cumulative exposure in internal analyses, unlagged and lagged for 10 and 20 years, with the highest risks among the highest exposure group. Cox regression analyses showed a positive association between cumulative exposure rank and urinary bladder cancer incidence in both categorical and continuous models of exposure, and an interaction was observed between exposure rank and age (with higher risk observed for workers under the age of 60). Sensitivity analysis of identification of cases supported the robustness of the results.

The NTP concluded that there is credible evidence of an association between increased urinary bladder cancer risk and exposure to *o*-toluidine, based on (1) consistent findings of elevated risk across the most informative studies, (2) a significant exposure-response relationship based on employment duration in both rubber chemical worker cohorts, (3) a significant exposure-response relationship based on cumulative exposure rank in both unlagged and lagged analyses of the NIOSH cohort, and (4) the large magnitudes of effect across studies. The negative results for the U.S. dye worker cohort study and the case-control study were attributed to their limited statistical power to detect an effect and to probable exposure misclassification.

Possible alternative explanations for the increased risk of urinary bladder cancer found in these studies were considered. In some of the studies, the small numbers of cases resulted in imprecise risk estimates; however, the 2013 update of the NIOSH cohort study was based on a larger number of cases, increasing the precision of the risk estimate for definitely exposed workers and the power to detect exposure-response relationships. The association is unlikely to be explained by selection bias or informational bias; although there was potential for non-differential exposure misclassification in some studies, the large magnitudes of the risk estimates mitigate concern. Tobacco smoking is also unlikely to explain the results. The NIOSH study found that in a subset of workers whose smoking status was known, smoking increased the risk of urinary bladder cancer only very slightly. The U.K. rubber chemical worker cohort study found no excess risk of lung cancer, and, again, the large magnitude of the risk estimates mitigates concern about confounding by smoking.

Potential confounding by occupational co-exposures also could be reasonably ruled out across the studies. In the NIOSH study of rubber chemical workers, co-exposures were well characterized. The most common co-exposure was to aniline; however, the exposure levels were much lower for aniline than for *o*-toluidine, and the subcohorts exposed to aniline did not show increased risks of urinary bladder cancer. In the U.K. rubber chemical workers exposed to *o*-toluidine, a significantly elevated risk of urinary bladder cancer was found after adjustment for co-exposure to aniline and other chemicals. Co-exposures are more of a potential concern in dye workers, who were exposed to some known animal carcinogens and other aromatic amines. However, the common exposure across the studies was to *o*-toluidine, while co-exposures varied among the cohorts, and there is no independent evidence that any of the co-exposures were to known human urinary bladder carcinogens.

Therefore, the NTP concluded that there is sufficient evidence of the carcinogenicity of *o*-toluidine from studies in humans. This conclusion is based on several epidemiologic studies that have found an increased risk of urinary bladder cancer among workers exposed to *o*-toluidine, which increased with increasing level or longer duration of exposure and was unlikely to be explained by chance, bias, or confounding.

IV.C.3.2 Peer Review Comments and Panel Discussion

Dr. Shelia Zahm, first reviewer, thought the literature search strategy (both search terms and sources) was comprehensive and exhaustive. The criteria for determining a study's relevance were sound and the assessments of study quality were excellent. The study summary tables were an extremely helpful way to present the study features; all types of potential biases were carefully evaluated and clearly described, including some biases rarely discussed in occupational studies. The descriptions of co-exposures and their possible impacts on the results were detailed and clear. The scientific information from the cancer studies was clear, technically correct, and objectively presented, and the evaluations were clear and objective.

Dr. Zahm noted apparent inconsistency between the information in Table 3-2 on "potential for confounding" and the statement on page 32 that the three dye worker studies handled co-exposures by exclusion. She agreed with the summaries, interpretation, and synthesis of the epidemiology studies. She approved of the inclusion of the section on "Summary of the utility of the studies to inform the cancer evaluation," and endorsed the selection of studies that were

considered adequate for inclusion in the evaluation. She also reviewed the 2013 update of the NIOSH study, which confirms, expands, and enhances the evidence for carcinogenicity. Since the last RoC review of *o*-toluidine, more epidemiologic evidence has accumulated with increasing certainty that urinary bladder cancer is excessive among exposed workers.

Regarding occupational co-exposures, the findings presented in the draft monograph establish that confounding by other occupational carcinogens cannot explain the huge risks found for *o*-toluidine. In the NIOSH study, there was good evidence that the association with *o*-toluidine was not confounded by exposure to aniline or 4-aminobiphenyl (4-ABP). With respect to potential confounding by non-occupational exposures, data on smoking in a subset of the NIOSH cohort indicate that confounding by smoking is not a reasonable explanation for the large urinary bladder cancer excesses observed. In summary, Dr. Zahm stated the studies were of sufficient quality for evaluation, that potential confounding could be ruled out as an explanation for the findings, and that the studies established *o*-toluidine as a urinary bladder carcinogen in humans.

Dr. Elizabeth Ward, second reviewer, agreed with Dr. Zahm's comments on the quality of the review and the strength of the science; however, she felt that potential confounding by 4-ABP could have been covered in more depth in the monograph. There was documented concern about 4-ABP in the early history of the plant and there is still potential for it to be generated.

Dr. Laura Beane Freeman, third reviewer, concurred with Drs. Zahm and Ward about the quality of the review of the human cancer studies. She commented that the observation of very large cancer risks across multiple exposure scenarios where *o*-toluidine was the common exposure supports the conclusion that the effects are due to exposure to *o*-toluidine, and not to some other unknown urinary bladder carcinogen.

Dr. Smith, fourth reviewer, agreed that review of the human cancer studies was excellent. He recommended that the Canadian population-based case-control study should not be included in Table 3-3 or in the cancer evaluation because the exposure assessment was quite inadequate; it used a U.S. job exposure matrix, with no exposure assessment in Canada. He also noted the inappropriateness of using a population-based case-control study to detect a rare occupational exposure. Dr. Smith did not give credence to potential confounding by tobacco smoking or 4-ABP, given the magnitude of the urinary bladder cancer risk observed for *o*-toluidine exposure. He also would not attach any importance to the age interaction observed in the updated analysis of the NIOSH cohort.

IV.C.3.3 Action

Dr. Zahm moved that the Panel accept the preliminary conclusion that there is sufficient evidence of the carcinogenicity of *o*-toluidine from studies in humans and that it is unlikely to be explained by chance, bias, or confounding. Dr. Ward seconded the motion, which passed unanimously (11 yes, 0 no, 0 abstentions).

IV.C.4 Metabolism and Mechanistic Data

IV.C.4.1 Presentation

Mr. Atwood presented an overview of the key information in the draft monograph sections on metabolism and mechanistic data. Aromatic amines include many genotoxic and carcinogenic compounds, subdivided into monocyclic compounds and bicyclic and polycyclic compounds. The data indicate that the monocyclic compounds share common target tissues, including the urinary bladder. The literature on the metabolism of *o*-toluidine is limited to a few studies in male rats, and there have been no definitive studies identifying the cytochrome P450 enzymes involved. Data in humans are limited to measurement of *o*-toluidine or its metabolites in the urine or of *o*-toluidine–Hb adducts in exposed workers.

An extensive genetic toxicology database exists for *o*-toluidine; the monograph therefore relies on reviews in the secondary literature. Much of the information on proposed mechanisms is based on analogies with bicyclic and polycyclic aromatic amines, for which there is an extensive database. Overall, the mechanistic data support the carcinogenicity of *o*-toluidine, which involves metabolic activation (for which hemoglobin adducts are biomarkers), DNA damage in several mammalian cell types (including human and rat urinary bladder mucosa), and oxidative DNA damage and cell proliferation.

Mr. Atwood described the key events in *o*-toluidine metabolism in the liver (N-hydroxylation, ring-hydroxylation, N-acetylation, and sulfate/glucuronide conjugation), blood (oxidized to *o*-nitrosotoluene, which is believed to be responsible for formation of methemoglobin and hemoglobin adducts), and urinary bladder epithelium (hydrolysis of glucuronides, O-acetylation, and oxidation of phenolic metabolites).

There is evidence that *o*-toluidine is genotoxic based on studies showing formation of DNA adducts, mutagenicity, DNA damage, and clastogenic effects. Although there are no structural criteria for predicting carcinogenicity of monocyclic aromatic amines, structural comparison studies have shown that N-oxidation is critical.

In response to a question from Dr. Sabbioni, Mr. Atwood noted that bulky *ortho* substitutions actually decrease mutagenic potency, whereas *ortho* substitutions of smaller groups (e.g., methyl or methoxy) can enhance genotoxicity and carcinogenicity.

IV.C.4.2 Peer Review Comments and Panel Discussion

Dr. Stephen Nesnow, first reviewer, stated that the section on disposition and toxicokinetics was technically correct, thorough, and objectively presented. It covered all the available data on the ADME of *o*-toluidine in humans and rodents and captured the complexity of the possible routes of metabolic activation of *o*-toluidine and the enzymes and reactive intermediates attributed to these processes.

Dr. Nesnow noted that in both humans and rodents, the urinary metabolite is exactly the same (*N*-acetyl-*o*-toluidine). Distribution data from one rat study showed that *o*-toluidine concentrations were highest in whole blood, spleen, kidneys, and liver. The metabolism is complex; there is not only ring-hydroxylation and N-hydroxylation, but also a small amount of C-hydroxylation, resulting in anthranilic acid. A number of enzymes have been suggested to be

involved, including CYP 1A1, 1A2, and 2E1 and the peroxidases. The key finding of *o*-toluidine–Hb adducts in male and female rats and in humans underscores the importance of *o*-nitrosotoluene as a toxicological intermediate (as a metabolite of *N*-hydroxy-*o*-toluidine). Dr. Nesnow agreed to provide an additional reference that shows induction of various enzymatic activities by *o*-toluidine. He agreed that a key issue is the ring hydroxylation leading to the quinones and iminoquinones, which can undergo redox cycling and generate ROS. It is a complex process involving both genotoxicity and ROS induction, which can also cause genotoxicity and cellular damage, leading to increased cell proliferation.

Dr. Nesnow noted that there is a wealth of data on the genotoxicity of *o*-toluidine, which is well covered in the monograph. He stated that the data in this section were clear, detailed, technically correct, and objectively presented. The data show that *o*-toluidine can bind to DNA and induce mutations and chromosomal damage.

He said there is no doubt that in a number of systems, including cultured rodent cells, *o*-toluidine induced DNA damage, strand breaks, chromosomal aberrations, aneuploidy, and cell transformation. In mice exposed *in vivo,* it induced sister chromatid exchange (SCE) in bone marrow and DNA damage (in the comet assay) in a whole series of tissues. Dr. Nesnow agreed to provide a paper not cited in the monograph that describes results of the comet assay in many tissues of mice and rats for a series of aromatic amines, including *o*-toluidine. In that study, *o*-toluidine caused DNA damage in stomach, liver, urinary bladder, lung, and brain tissue. In rats, *o*-toluidine induced micronuclei in peripheral blood lymphocytes and DNA damage in stomach, colon, kidney, and urinary bladder tissues. Thus, there is consistency in the results for DNA damage in the urinary bladder.

Dr. Nesnow noted a correction in the description of the results of Watanabe *et al.* (2010); copper is not required for induction of 8-oxodG adducts in human leukemia cells by *o*-nitrosotoluene. He will provide a paper not cited that describes induction of free radicals in yeast by *o*-toluidine. He suggested adding a table showing the tumor sites for related chemicals, such as *o*-nitrotoluene and *o*-nitrosotoluene. He also suggested adding cell transformation to the list on page 79 of effects induced by *o*-toluidine.

Dr. Sabbioni, second reviewer, found it confusing that the monograph discussed metabolism and disposition separately from mechanistic data. He suggested that the discussion of *o*-toluidine metabolism in humans should start with a statement that *o*-toluidine was found unmetabolized in human urine. He also noted that Gaber *et al.* (2007) is not the correct source for the statement that *o*-toluidine is a major metabolite of prilocaine; he suggested citing instead the NTP (2000) report. He suggested that the monograph should cite the original sources of the information, not just newest literature.

Dr. Sabbioni said that Table 5-1 should not show positive results for DNA adducts in humans exposed *in vivo* and that the mention of *o*-toluidine–releasing DNA adducts in humans should be removed from the monograph. He noted that Skipper *et al.* (2010) does not mention *o*-toluidine.

Dr. Nesnow commented that because the paper that reports *o*-toluidine–releasing DNA adducts in humans (Böhm *et al.,* 2011) was published in a peer-reviewed journal and reports a key finding, it needs to be cited, but with qualifying statements about the methodological concerns.

Dr. Talaska noted that the finding of DNA adducts in rat liver but not the urinary bladder is consistent with data for aromatic amines. In the synthesis of mutagenicity assay results, the "special protocols" mentioned have been in use for 25 years and are not unusual. Dr. Talaska said that it was very unlikely that unconjugated *N*-hydroxy metabolites of aromatic amines would enter the urinary bladder lumen; he noted that in the study cited (Kadlubar *et al.,* 1991), the animals were exposed via intravesical instillation, and no free *N*-hydroxy metabolites were found in the blood even at very high doses. He suggested revising the last line on page 76 by changing "*N*-hydroxy-*ortho*-toluidine and its conjugates…" to read "*N*-hydroxy-*ortho*-toluidine conjugates…." He agreed to provide references documenting that DNA adducts of aromatic amines have caused chromosomal damage directly.

Dr. Sandy agreed with Dr. Nesnow's statement about oxidative damage also potentially leading to oxidative DNA damage. This should be added to the discussion of redox cycling, cytotoxicity, and cell proliferation. She suggested changing the title of Table 5-1 to reflect the fact that it includes non-genetic effects, or else reporting the non-genetic effects in a separate table. She acknowledged that the RoC monographs are not meant to be encyclopedic, and suggested that the RoC staff needs to find a balance between citing original studies and citing recent reviews.

IV.C.5 Overall Cancer Evaluation

IV.C.5.1 Presentation

Dr. Lunn presented an overview of the overall cancer evaluation. The preliminary listing recommendation was that *ortho*-toluidine is *known to be a human carcinogen* based on increased risks of urinary bladder cancer among *o*-toluidine–exposed workers together with cancer studies showing site concordance for urinary bladder cancer in female and male rats and humans and mechanistic data demonstrating biological plausibility in humans.

The proposed mechanism for urinary bladder carcinogenicity is similar to that of other aromatic amines that cause urinary bladder cancer in humans. Metabolic activation results in binding of reactive metabolites to DNA and proteins, oxidative DNA damage, and genotoxicity. Data suggesting that the key metabolic steps and genotoxic effects occur in humans include the observation of *o*-toluidine–Hb adducts in humans and genotoxicity in human lymphocytes and urinary bladder mucosal cells.

IV.C.5.2 Peer Review Comments and Panel Discussion

Dr. Sabbioni, first reviewer, said that the overall cancer evaluation was well done; he had nothing to add except that reference to DNA adduct formation in humans should be removed.

Dr. Ward, second reviewer, agreed that the weight of the evidence for human carcinogenicity is very strong and that, for the most part, the genotoxicity and mechanistic information supports the experimental animal and human cancer data.

Dr. Sandy also agreed with the overall cancer evaluation. Dr. Zahm suggested that the wording in the preliminary decision be modified to that it is based on sufficient evidence from studies in humans together with "cancer studies in animals, including site concordance," because site concordance itself is not a requirement for sufficient evidence of carcinogenicity in animal

studies. Drs. Sandy, Ward, and Talaska agreed with the suggestion. Dr. Nesnow said that some of the information he had suggested adding to other monograph sections should also be added to the overall cancer evaluation, including mention of C-hydroxylation in *o*-toluidine metabolism and cell transformation as a genotoxic effect of *o*-toluidine.

IV.C.5.3 Action

Dr. McMartin asked the Panel to vote on the recommendation that *o*-toluidine be listed in the RoC as a *known to be a human carcinogen* based on increased risks of urinary bladder cancer among *o*-toluidine exposed workers in concert with cancer studies in animals, including site concordance for urinary bladder cancer in female and male rats and humans, and mechanistic data demonstrating biological plausibility in humans. Dr. Talaska moved to approve the recommendation. The motion was seconded by Dr. Ward and passed unanimously (11 yes, 0 no, 0 abstentions).

IV.D. Draft RoC Substance Profile

Dr. Lunn summarized the contents of the draft substance profile as containing NTP's preliminary listing status recommendation, summarizing the scientific information key to reaching a recommendation, and providing information on properties, use, production, exposure, and existing federal regulations and guidelines. She noted that any changes made in revising the draft monograph would carry over into the draft substance profile.

IV.D.1.1 Peer Review Comments and Panel Discussion

Dr. Talaska, first reviewer, stated that the profile was generally a good review and condensation of the important data. He suggested that the discussion of exposure should include second-hand smoke and an explanatory clause to the effect that the impact of occupational exposure is much greater than that of smoking. Dr. Zahm, second reviewer, said that the highlighted information from the cancer studies in humans includes everything that is key to reaching the listing recommendation; she had nothing to add beyond the changes already suggested. Dr. Sandy, third reviewer, suggested mentioning the mammary-gland adenocarcinoma seen in male rats.

Dr. Nesnow, fourth reviewer, said the discussion in the profile on mechanistic and genotoxicity data summarized the salient findings presented in the monograph, integrating both direct and indirect evidence on *o*-toluidine and providing a plausible summary of the information. It related its findings to known mechanisms of urinary bladder cancer previously determined with other aromatic amines and drew linkages to those mechanisms for *o*-toluidine. He agreed with the monograph's conclusions on the mechanisms of carcinogenicity of *o*-toluidine. However, he felt that some information on absorption and distribution was missing; the human and rodent urinary metabolite *N*-acetyl-*o*-toluidine should be mentioned, as well as tissue distribution in rodents. The information on induction of 8-oxodG adducts in human leukemia cells by *o*-nitrosotoluene should be corrected and cell transformation should be added as another genotoxicity end point. Dr. Nesnow clarified that cell transformation can occur through both genotoxic and non-genotoxic mechanisms.

Dr. Sabbioni noted that the reference to *o*-toluidine–releasing DNA adducts in humans needs to be addressed. He said the *N*-acetyltransferase reaction is not the only mode of action for DNA adduct formation; the reaction can also occur without *N*-acetyltransferase if the urine is acidic enough; therefore, this discussion should be expanded. Dr. McMartin wondered whether the statement about *N*-acetyltransferase not binding strongly to *o*-toluidine was relevant, because the binding would occur with the *N*-hydroxy metabolite. Dr. Paul Villeneuve suggested adding language to the monograph to describe why less emphasis should be placed on the Canadian case-control study.

Dr. Sabbioni left the Panel meeting following completion of the *o*-toluidine draft monograph review.

V. Draft RoC Monograph for Pentachlorophenol and By-products of Its Synthesis

V.A. Oral Public Comments

Dr. Robert Golden, ToxLogic LC, commented by telephone on behalf of the Pentachlorophenol Task Force. He noted that Dr. Elizabeth Delzell was involved in preparing the epidemiological comments. Dr. Golden commented that although 2,3,7,8-tetrachlorodibenzodioxin (TCDD) is not considered a by-product of pentachlorophenol synthesis for the purpose of listing in the RoC, potential confounding by this known human carcinogen is a concern in studies from Europe, New Zealand, and the U.S. He questioned the validity of listing pentachlorophenol when a potential contaminant known to have similar effects already is listed. He mentioned that non-Hodgkin lymphoma (NHL) was the major cancer site of interest.

Dr. Golden questioned the validity of the RoC review process and clarity of the terms *sufficient evidence*, *causal relationship*, *limited evidence*, *causal interpretation*, and *adequately excluded*. He questioned the validity of basing conclusions about human carcinogenicity solely on data from studies in experimental animals. He stated that assessment of the potential carcinogenicity of pentachlorophenol should be based on multiple human studies and that the only relevant framework for the review is the 2013 NTP Office of Health Assessment and Translation (OHAT) Draft Approach for Systematic Review and Evidence Integration for Literature-Based Health Assessments.

Dr. Golden stated that unexplained inconsistency should reduce confidence in the body of evidence for carcinogenicity of pentachlorophenol, and that none of the factors that would increase confidence (large magnitude of effect, dose response, consideration of all plausible confounding, and consistency across species, populations, and studies) are applicable to the data for pentachlorophenol. He noted that the OHAT Approach embraces the Hill considerations for determining causation, but they do not appear to have been used in reaching the final conclusion on pentachlorophenol.

Dr. Golden provided an overview of the key epidemiological studies of pentachlorophenol and NHL identifying potential confounders. He stated that even in studies where it was assumed that there was no TCDD exposure, there actually was TCDD exposure in some cohorts, so one could not be confident that an increase in NHL was not due to TCDD exposure. He identified

the Demers *et al.* (2006) cohort study as the only study without confounding co-exposures and with a statistically significant exposure-response relationship.

Dr. Golden then reviewed factors related to biological plausibility of mechanisms of carcinogenesis. He stated there is little *in vivo* evidence for pentachlorophenol-induced genotoxicity, and that carcinogenesis of pentachlorophenol in animals likely involved high-dose cytotoxicity, via oxidative stress, ROS-induced DNA damage or mutations, inhibition of gap junction intercellular communication, and chronic inflammation. Dr. Golden considered the two-year rat study with 99% pure pentachlorophenol to be the most relevant for potential effects in humans, but the full study found no pentachlorophenol-related tumors in either sex at any dose.

Dr. Golden's final conclusions were: (1) no significant finding in any epidemiology study is corroborated in a different study; (2) the RoC listing criteria are not satisfied for either *known* or *reasonably anticipated to be a human carcinogen* by the available human and animal data for pentachlorophenol; (3) based on the key scientific questions stated in the draft RoC monograph, the level of evidence from human studies for the carcinogenicity of pentachlorophenol is *limited*; and (4) based on the NTP OHAT approach, pentachlorophenol would be classified as *suspected of carcinogenic potential*.

V.B. Scientific Issues in Written Public Comments

Dr. Wolfe said the NTP had received written comments on behalf of the Pentachlorophenol Task Force from Drs. Golden and Delzell and Dr. Herbert Estreicher, which were provided to the Panel to be considered in their review and were posted to the NTP website. She identified the following major scientific issues raised in the public comments:

- Neither pentachlorophenol nor by-products of its synthesis are carcinogenic; therefore, the commenters disagreed that pentachlorophenol and by-products of its synthesis are *known to be a human carcinogen*.
- The commenters disagreed with the conclusion in the monograph that there is *significant exposure*. They stated that (1) current uses do not lead to significant exposures, (2) detection of pentachlorophenol in urine does not definitively indicate exposure to only pentachlorophenol because it is also a metabolite of other chemicals, (3) the presence of higher-chlorinated dioxins in populations near wood treatment facilities could be the result of their continued presence in soil from the past, and (4) exposure to pentachlorophenol has decreased over time due to restricted use and regulations.
- The commenters questioned the role of TCDD in the listing decision, on the grounds that TCDD should not be considered a by-product of pentachlorophenol synthesis in the U.S., that it should be treated as a confounder in the human studies, and that it is already recognized as a known carcinogen by IARC.
- Biological plausibility is not adequately addressed in the evaluation.

The commenters considered the epidemiologic evidence pertaining to the carcinogenicity of pentachlorophenol in humans as inconclusive and limited. They stated that the studies cited in the draft monograph do not indicate there is a valid, strong, and consistent association between exposure to pentachlorophenol and NHL or other forms of cancer. Clear evidence of

a monotonic exposure response is lacking, and explanations provided for the lack of trends are speculative.

Regarding the animal evidence, they stated that 99% pure pentachlorophenol and by-products of its synthesis are not carcinogenic. They questioned some of the animal data's relevance to human cancers and considered the negative *in vivo* genotoxicity studies as showing a lack of biological plausibility for mutations playing a role in cancer induction by pentachlorophenol.

V.C. Draft Cancer Evaluation Component

V.C.1 Properties and Human Exposure

V.C.1.1 Presentation

Dr. Gloria Jahnke presented an overview of the key information in the properties and human exposure section of the draft monograph. Pentachlorophenol was selected as a candidate substance because of evidence of widespread past and present exposure from its ubiquitous commercial and residential use as a wood preservative and multipurpose biocide from 1936 to 1984. Since 1987, its use has been restricted; it is now registered only for wood preservation and restricted to commercial use (i.e., for utility poles and cross arms, railroad ties, laminated wood, and wharf pilings). Numerous studies of pentachlorophenol in experimental animals and humans have been published, as well as some reviews. Since the 1999 IARC review of chlorophenols, which found limited evidence for carcinogenicity in humans, several cohort studies of pentachlorophenol exposure have been published. The U.S. EPA Integrated Risk Information System (IRIS) report on pentachlorophenol (not including by-products of its synthesis) concluded that it is *likely carcinogenic to humans* based on strong evidence for NHL and multiple myeloma, and additional cohort studies have since been published.

In preparing the draft monograph on pentachlorophenol and by-products of its synthesis, NTP relied on two forums to provide information on differentiating potential carcinogenic effects of pentachlorophenol exposure from effects due to occupational co-exposures or to pentachlorophenol contaminants. In a webinar held on human epidemiological studies, input was received from the public and from experts in the field; discussions of what chemicals people are exposed to led to clarification of the candidate substance. In a government informational group on cancer studies in experimental animals, government toxicologists and dioxin experts discussed the potential effects of pentachlorophenol and individual by-products and the cumulative effect of dioxin-like by-products.

These discussions led to the candidate substance being defined as *pentachlorophenol and by-products of its synthesis*. By-products of synthesis make up 10% of commercial (technical-grade) pentachlorophenol; these include the tri- and tetra-chlorinated phenols, hexachlorobenzene, and dioxins and furans (primarily hexa-, hepta-, and octa- dioxins and furans). TCDD is considered to be a contaminant, as it is not produced by the pentachlorophenol synthesis method used in the U.S., and it is rarely detected in commercial pentachlorophenol preparations.

A large cancer study database exists that includes studies of exposure to pentachlorophenol and its by-products. Also, previous pentachlorophenol exposure can be assessed from blood

levels of the long-lived hexa-, hepta-, and octa-dioxin congeners. Collins *et al.* (2008) found that dioxins measured 20 years after exposure differed between production workers exposed only to pentachlorophenol and production workers exposed to trichlorophenol (TCP); the higher chlorinated dioxins occurred at high levels in the pentachlorophenol workers (constituting a "pentachlorophenol dioxin fingerprint"), while TCDD (a by-product of TCP synthesis) was predominant in the TCP workers. Other studies have found high levels of the same higher chlorinated dioxins (and very low levels of TCDD) in people or animals exposed to pentachlorophenol occupationally across different geographical locations and occupations and exposed environmentally (i.e., people living near wood treatment plants and cattle in feedlots with pentachlorophenol -treated poles). Virtually everyone who is exposed to pentachlorophenol is also exposed to its by-products.

It was concluded that a significant number of people living in the U.S. are exposed to pentachlorophenol and by-products of its synthesis. Exposure is lower now than in the past, but exposure to workers and the general public still occurs. Evidence of recent exposures comes from the dioxin fingerprint in the blood of people living near wood treatment facilities; environmental and biological samples from preschool children and their homes and daycare centers; data from the National Health and Nutrition Examination Survey (NHANES); findings of low levels of pentachlorophenol in foods, water, air, dust, and soil; TRI data indicating release of pentachlorophenol from 30 U.S. facilities in 2011; and the presence of pentachlorophenol at Superfund sites. The general population is exposed primarily by inhalation and ingestion. Occupational exposure occurs among workers who formulate pentachlorophenol for use or who treat lumber or come into contact with treated lumber. Pentachlorophenol is found in the urine of workers from wood-treatment plants, and the pentachlorophenol dioxin fingerprint is found in the blood of former pentachlorophenol production workers. Exposure from treating lumber is primarily dermal, but pentachlorophenol processing and pressure-treatment of wood can result in inhalation exposure.

Dr. Talaska asked whether urinary or blood pentachlorophenol levels were also available for the people whose dioxin congener levels were reported by Collins *et al.* (2008). Dr. Lunn said pentachlorophenol levels were not available; the workers were classified as pentachlorophenol or TCP production workers based on a job classification matrix, and serum levels of dioxins were measured 20 years after exposure. Dr. Beane Freeman asked whether TCDD might be a by-product of pentachlorophenol production by processes used outside the U.S. Drs. Lunn and Jahnke said that although this is possible, the data have not shown elevated TCDD levels in non-U.S. pentachlorophenol production workers. Dr. Smith noted that the pentachlorophenol manufacturing process in New Zealand is the same as that used in the U.S. Dr. Jahnke said that TCDD can, however, appear as a contaminant of pentachlorophenol. In response to a question from Dr. Talaska, Dr. Jahnke said she did not have figures on how much pentachlorophenol is imported from China. Dr. McMartin suggested adding information on imports to the monograph.

V.C.1.2 Peer Review Comments and Panel Discussion

Dr. Talaska, first reviewer, suggested that if U.S. imports of pentachlorophenol from China are increasing, the monograph should also indicate whether increasing TCDD contamination might

be anticipated. Dr. McMartin suggested that "toxic equivalents" should be defined in the monograph (in terms of the toxic effect being compared). Dr. Talaska suggested that the U.S. Department of Transportation number be added to Table 1-1 and the equilibrium concentration to Table 1-2. More emphasis should be placed on the ACGIH and OSHA skin notations for pentachlorophenol, and it should be noted that the TLV documentation mentions that dermal exposure to pentachlorophenol has resulted in a number of fatalities.

Although the majority of pentachlorophenol (~80%) is eliminated unchanged, ~20% is eliminated as a metabolite. The monograph should mention that there is an ACGIH biological exposure index for pentachlorophenol in urine, and that it indicates that hydrolysis should be used to obtain total pentachlorophenol. Dr. Talaska noted some inconsistencies in the monograph regarding metabolic pathways in humans and in NHANES data. He suggested the monograph include more information on possible trends in pentachlorophenol air levels and urine levels, on annual production of telephone poles and crossties, and on whether widespread exposure in the U.S. is due to pentachlorophenol's presence *and persistence* in the environment.

Dr. Stelvio Bandiera, second reviewer, found the description of the chemical identity and properties of pentachlorophenol to be quite well written. It would be useful to add a table listing the by-products of each of the two routes of pentachlorophenol synthesis. For the indirectly exposed general population, the main route of exposure is presumably in the diet. He said pentachlorophenol and some of the other chlorinated dioxins and furans are legacy chemicals that persist for years in the food chain. The dietary exposure is not just to pentachlorophenol, but to a large range of other long-lived chlorinated compounds; the cumulative exposure to this conglomerate of chemicals is different for indirect vs. direct exposure. Dr. Bandiera objected to the use of the term "half-life" to describe changes in global pentachlorophenol concentrations in human urine. He said biomonitoring data reflect exposure and do not equate to biological or elimination half-life. He also suggested adding information on the number of people who are occupationally exposed to pentachlorophenol, if only approximate.

Dr. Smith questioned whether there was any information on the range and distribution of by-product concentrations in commercially available pentachlorophenol in the U.S. and whether or how their levels have changed over the years. Mr. Kevin Dunn stated that historically, levels of by-products increased or decreased over the years, but were always present and did not exhibit a trend. Dr. Smith suggested that the data be added to the monograph. Mr. Dunn also responded to Dr. Talaska's earlier question by stating that there is no evidence that pentachlorophenol is currently imported into the U.S. from anywhere but Mexico.

Dr. McMartin suggested that the exposure section of the monograph include definitions of the various pentachlorophenol products and list the other components of technical-grade pentachlorophenol. Dr. Jahnke said that the information is available and can be added. Dr. McMartin also asked the Panel to address the question of whether current uses of pentachlorophenol lead to significant exposure. Dr. Talaska stated that there is residual exposure in the general population, and that a number of people still work manually with treated wood.

Dr. McMartin summarized the general sense of the Panel as in agreement that a significant number of people living in the U.S. are (or were) exposed to pentachlorophenol and by-products of its synthesis.

V.C.2 Cancer Studies in Humans

V.C.2.1 Presentation

Dr. Jennifer Ratcliffe presented an overview of the key information in the draft monograph section on human cancer. She described the studies identified in the literature search and the rationale for their inclusion/exclusion. For the purposes of the evaluation, the cancer end points of concern were NHL, multiple myeloma, soft-tissue sarcoma, kidney, liver, and lung cancer, and all cancers combined.

The two most informative studies were considered to be the cohort study of Canadian sawmill workers (Demers *et al.,* 2006) and the cohort study of U.S. (Michigan) pentachlorophenol producers (Ramlow *et al.,* 1996, Collins *et al.,* 2009). The Canadian sawmill workers study was extremely large (over 27,000 workers), with individual work history and industrial hygiene data. Exposure was about 95% dermal, and it was possible to estimate and validate cumulative dermal exposure to pentachlorophenol and tetrachlorophenol (TeCP). The study of Michigan pentachlorophenol producers identified about 770 workers for which mortality data were analyzed. Industrial hygiene data allowed derivation of individual work histories. Some of the workers were co-exposed to TCP, and the study assessed cumulative exposures to pentachlorophenol, pentachlorophenol dioxin by-products, and total dioxin toxic equivalents.

Less informative studies on NHL were the NIOSH pentachlorophenol producers cohort study (Ruder and Yiin, 2011), the nested case-control study of dioxin exposures, and the Swedish population-based case-control studies. The NIOSH pentachlorophenol producers study overlapped the Michigan pentachlorophenol producers study; about a third of the workers in the NIOSH study were from the Michigan pentachlorophenol plant. Dr. Ratcliff provided further comparisons of the Michigan and NIOSH studies and addressed the public comment concerning the discrepancy in the number of TCP exposure workers reported for the Michigan pentachlorophenol production workers by the two overlapping studies. Using information included in the monograph and information obtained through personal communications with the study authors, she explained that the two studies used different methods of characterizing TCP exposure.

Next, Dr. Ratcliffe discussed the findings. The strongest evidence of an association with pentachlorophenol exposure is for NHL. Increased risks of NHL were found in all studies, but the strength of the association varied across studies. The strongest evidence was from the Canadian sawmill workers cohort study, supported by the Michigan pentachlorophenol producers cohort study. A significant exposure-response relationship with dermal exposure to pentachlorophenol but not TeCP was observed in the Canadian sawmill cohort study. Another analysis of this cohort was published by Friesen *et al.* (2007), who looked at the relationship between continuous cumulative pentachlorophenol exposure and relative risk (RR) of NHL. With a 20-year lag, they found an almost monotonic exposure-response relationship (based on log-linear and log-log relationships).

In the Michigan pentachlorophenol producer cohort study, the highest risk of NHL was seen among the workers with the highest blood levels of dioxin by-products of pentachlorophenol synthesis. No exposure-response relationship was observed; however, there was potential for misclassification of exposure in the lower exposure groups due to the complexity of dioxin modeling. No internal analyses were conducted for pentachlorophenol by-products

Evidence from the other studies was more limited but collectively supported the findings of the more informative studies. A non-statistically significant increased risk of NHL mortality was seen in the NIOSH pentachlorophenol producers cohort study, and no exposure response relationship was observed in either external or internal analyses. Increased risks of NHL were also observed in the Swedish case-control studies (Hardell *et al.,* 1994, 2002).

The associations between pentachlorophenol exposure and NHL in the cohort and nested case-control studies are unlikely to be explained by selection or information bias because individual work histories and industrial hygiene data were used. There is potential for non-differential exposure misclassification in the case-control studies, but attempts to obtain information from employers and the use of detailed questionnaires somewhat lessened that concern for the Swedish studies. A subanalysis of smoking in the Canadian sawmill worker cohort study found little effect on any endpoint. Other NHL risk factors were considered unlikely to be related to pentachlorophenol exposure.

With respect to occupational co-exposures, TeCP (which did not contain TCDD as a contaminant) showed no clear association with NHL in the Canadian sawmill workers cohort study; however, no independent evidence is available for evaluation of the carcinogenicity of TeCP. No other wood preservatives (such as creosote or copper chromate arsenate) were used in these sawmills, and other potential co-exposures (e.g., wood dust) are not known risk factors for NHL. In the Michigan pentachlorophenol producers cohort study, no association was observed between NHL and cumulative exposure to TCDD among all TCP workers. Other exposures in the cohort studies did not include known risk factors for NHL. In the case-control studies, there was potential for confounding by co-exposures to phenoxy herbicides or other chlorophenols.

There was some evidence for associations of pentachlorophenol exposure with multiple myeloma and kidney cancer. Significant exposure-response relationships were found in the Canadian sawmill workers cohort study for both of these cancers, as well as non-significantly elevated risks in the other cohort studies. Findings across studies were conflicting for soft-tissue sarcoma (a very rare cancer). Little or no evidence was found for associations between pentachlorophenol exposure and liver or lung cancer (which were examined only in the cohort studies). The NIOSH pentachlorophenol producers cohort study also found an elevated risk for all cancers combined.

The NTP's preliminary conclusion is that there is sufficient evidence for the carcinogenicity of pentachlorophenol and by-products of its synthesis from studies in humans, based on:

- Consistent evidence of an association between pentachlorophenol and by-products of its synthesis and non-Hodgkin lymphoma across studies
  - Different populations, geographical areas, and study designs
  - Strength of the association varied across studies

- Highest risk among those with the highest exposure
  - Exposure response relationship observed with cumulative dermal exposure in most informative study
  - Higher risk observed among pentachlorophenol producers with the highest level of pentachlorophenol by-products
- Not reasonably explained by chance, bias or confounding
- "Dioxin-like activity" of pentachlorophenol by-products of synthesis may contribute to the carcinogenicity

Dr. Smith requested clarification on the plotting of the exposure-response graph from Friesen *et al.* (2007) because it differed from the graph in the published paper.

V.C.2.2 Peer Review Comments

Dr. Smith, first reviewer, thought the draft monograph gave a good summary of the epidemiological information in each of the relevant studies and considered the approach systematic and transparent. However, inadequate attention was given to the scientific plausibility of findings in various studies, especially in relationship to the degree of exposure.

Regarding the Swedish case-control studies, Dr. Smith did not consider the exposure assessments to have been validated. Further, he considered a number of the study results implausible, e.g., the high RR estimate of 8.8 for exposure of just one month or more (compared to a RR of just 1.71 for exposure of five years or more in the Canadian study). Additionally, the upper confidence interval (CI) limit in the Canadian sawmill worker cohort study (3.24) was much lower than the *lower* confidence interval limit for the Swedish case-control study. Dr. Smith provided other examples of outlier results reported by these investigators for very short exposure periods and recommended more critically assessing their findings.

Dr. Smith recommended not relying on the data from any of the case-control studies (except for the case-control study nested in a cohort) to support conclusions about increased risks from pentachlorophenol exposure. Therefore, the evaluation should focus on the cohort studies, which Dr. Smith found to be well presented, though he had some points to make about each study.

In the Canadian sawmill workers cohort study, which Dr. Smith considered to be the strongest study, 12 incidence cases produced a RR of 1.98 (with a CI of 0.97-4.06), which he did not consider a strong association. To support a relative risk of about 2, Dr. Smith suggested that five or ten confirmatory studies are necessary. The test for trend had a *P* value of 0.02, but based on his reading of Friesen *et al.,* the trend was not monotonic. He thought these findings needed more careful consideration.

In the NIOSH pentachlorophenol producers cohort study, those workers exposed to pentachlorophenol with no TCP exposure, had a standardized mortality ratio (SMR) for NHL of 1.41 (with a lower CI limit of 0.64). For those exposed to both pentachlorophenol and TCP, the SMR was higher (2.5, with a lower CI limit of 1.08). These data, presented in Table 3-4 of the draft monograph, should be further analyzed. For pentachlorophenol alone, the CI is very wide, providing very weak evidence for pentachlorophenol and pointing to TCP (with TCDD as

contaminant) as possibly related to NHL. The trend by duration of pentachlorophenol exposure provides no evidence supporting causal association, but is actually inconsistent with a causal association. In Dr. Smith's opinion, the NIOSH study detracts from the evidence that pentachlorophenol causes NHL.

In the Michigan pentachlorophenol producers cohort study, NHL mortality was increased (SMR = 2.8) in pentachlorophenol workers without TCP exposure. The strongest finding was an SMR of 4.5, but this was based on 4 deaths (with a lower CI limit of 1.2) related to exposure to toxic equivalents of TCDD. This study provides some evidence of a relationship between NHL and the manufacture of pentachlorophenol, but the data suggest that it might be a consequence of high exposure to certain dioxins in the manufacturing setting, rather than exposure to pentachlorophenol itself. Dr. Smith said he found it impossible not to think of pentachlorophenol separately from its by-products and dioxin contaminants, and he considered it dangerous to consider them together. He was troubled by the idea of labeling a substance together with its by-products. He did not think that the Michigan producers cohort study supported the carcinogenicity of pentachlorophenol alone.

Dr. Smith noted that Kogevinas *et al.* (1995) had the strength of being a case-control study nested in a worker cohort (not the general population). The estimated OR for pentachlorophenol was 2.75, but the lower CI limit was 0.45, so he did not consider the study informative.

Dr. Smith's conclusion in considering the four cohort studies was that the epidemiological evidence for an association between pentachlorophenol exposure and NHL is limited.

Dr. Villeneuve, second reviewer, stated that he shared many of the same views expressed by Dr. Smith. Generally, he liked the structure of the section and the description of the literature search methods, and thought that the key studies were identified. However, given the number of case-control and cohort studies available, he would have excluded the cross-sectional ecological study immediately. He questioned the exclusion of the McLean *et al.* (2007) study on New Zealand workers. The study was limited by the small sample size for NHL, but it showed a reduced risk.

Dr. Villeneuve said the monograph identified common and not-so-common sources of bias, but he found this section hard to read, because each source of bias was identified and then evaluated in every study. He thought the discussion might have been less disjointed if each study were reviewed with respect to all sources of bias. He suggested grouping all of the childhood cancer studies together, as was done for other cancer end points. He cited Section 3.1.1, "none of the available cohort studies conducted multivariable analyses in which co-exposures or other potential confounders were considered." He considered this an important point, and given this limitation, found it hard to come to the conclusion that there is sufficient evidence for the carcinogenicity of pentachlorophenol.

Dr. Villeneuve agreed that the Canadian sawmill workers cohort study was the strongest study. However, he noted that the positive associations were found in the internal cohort analysis, and that none of the external analyses revealed any statistically significant association. Given that in discussion of some of the other studies, significant excess risks in external analyses were highlighted, this treatment could appear unbalanced. The Canadian sawmill workers cohort

study also indicated a significantly reduced risk of soft-tissue sarcoma (by about five-fold). Dr. Villeneuve concurred with Dr. Smith's comments about the Swedish case-control study regarding lack of exposure assessment validation and lack of credibility.

Dr. Villeneuve considered the epidemiological evidence for carcinogenicity of pentachlorophenol to be limited, because the results of the studies are mixed, confounding may not be adequately taken into account, and risks are typically based on very small numbers of cancers.

Dr. Zahm, third reviewer, suggested that the literature search should have included "polychlorophenol" (as used by IARC) as a search term. She also noted some inconsistency in the initial inclusion or exclusion of papers presenting data on chlorophenols as a group only. Specifically, she wondered why the studies by Smith were mentioned, but not similar studies by Partanen, Garabedian, and Hoppin that were cited by Dr. Paul Demers in his presentation at the NTP webinar.

Dr. Zahm characterized her view of the literature as being more "glass half full" than that of the previous reviewers. She considered the Canadian sawmill workers cohort study to be quite strong, with a large population, showing an association with NHL based on incidence, not just mortality, and with very little evidence for confounding by TCDD or TeCP. The other studies were all small; nonetheless, they showed some statistically significant results and consistency, and they were supportive to the extent they could be, given their small size. Dr. Zahm acknowledged that the body of literature was not large, consisting of one excellent study and other very small studies that were somewhat supportive.

Dr. Beane Freeman, fourth reviewer, agreed that the Canadian sawmill workers cohort study was clearly the strongest study, with respect not only to size, but also to exposure assessment and lack of evidence for confounding by occupational co-exposures; it showed what she would categorize as a moderate association between pentachlorophenol exposure and NHL. The fact that the NIOSH pentachlorophenol producers cohort study showed a stronger risk when workers exposed to TCP were included in the analysis suggested to her that the effect could be attributed to TCP, rather than pentachlorophenol. She noted that the large differences in classification of the same workers in the NIOSH and Michigan studies indicated considerable uncertainty as to the nature of the exposures, and it is hard to say that the increased risk was not due to TCP exposure. The Swedish case-control studies are not very informative, based on the exposure assessment. She found the preliminary recommendation in the monograph (Section 3.6) to be a bit confusing because it first mentions pentachlorophenol and the by-products of its synthesis and then mentions the carcinogenicity of TCDD as an example of the kind of activity that might relate to the NHL risk. She said the statement that "dioxin-like activity" of the by-products of pentachlorophenol synthesis may contribute to carcinogenicity sounds more like hypothesis than something that is supported by the human cancer studies.

V.C.2.3 RoC Staff Response and Panel Discussion

Concerning the issue of consistency in reliance on internal or external analyses, Dr. Lunn noted that in the Canadian sawmill workers cohort study, the external analysis was for the total cohort of sawmill workers and was not specific to pentachlorophenol, whereas the internal analysis was specific to pentachlorophenol. In the Michigan pentachlorophenol producers cohort study,

the metric considered most relevant was the dioxin fingerprint for pentachlorophenol exposure, because the candidate substance was defined as including the by-products of pentachlorophenol synthesis. However, there was no internal analysis for the dioxin fingerprint, only an external analysis. There was an internal analysis for total dioxin toxic equivalents, which was considered less informative for evaluation of pentachlorophenol and by-products of its synthesis. Dr. Lunn agreed that the discrepancy in exposure classification between the two pentachlorophenol producers cohort studies would decrease confidence in the results. However, it would not affect the results of the dioxin fingerprint analysis, which was not based on the TCP exposure classification.

Dr. Lunn appreciated the comments on exposure assessment in the Swedish case-control studies. She said it raised an important point about the evaluation of study methodology, which considered all possible types of bias, but never asked the question, "Does it make sense?" Dr. Smith said he thought the problem with the Swedish studies was most likely related to interview and recall bias, but could not prove it. Dr. McMartin noted that in the public comments, it was stated that the OR of 8.8 found in the Swedish case-control study declined to 1.2 in a later study by the same investigators; he asked whether the second study was of the same workers. Dr. Smith said the Swedish investigators did two separate studies and combined them in some papers but not others. Dr. Lunn said that for the evaluation, an attempt was made to use studies that did not overlap, based on recruitment dates. Dr. Ratcliffe said the second study differed in covering a broader area of Sweden and a different calendar time period.

Dr. Ward asked whether differences in the results between the sawmill worker and pentachlorophenol producer cohort studies were related to different exposure circumstances, as exposure of the sawmill workers was almost entirely dermal. Dr. Ratcliffe said no information was available on how pentachlorophenol production workers were exposed, but that it presumably differed from the exposure of sawmill workers. Data for comparing urinary pentachlorophenol levels or the dioxin fingerprint among the studies are not available. Concerning exclusion of the non-peer-reviewed study of New Zealand sawmill workers, Dr. Lunn said the study was well designed and had been externally reviewed. However, the total number of cancers was only 20, so even if it had been published, it would not have contributed to the evaluation.

Dr. Smith noted that the statement in the written public comments that IARC classified of dioxin as a known carcinogen "with emphasis on NHL" is incorrect; the classification was not based on NHL. In reference to the issue of information bias raised in the public comments, Dr. Zahm said that the Panel did not have any concerns about information bias in the studies that are critical for the evaluation.

Dr. Bandiera asked that the specific by-products included in the candidate substance be provided to the Panel.

V.D. Closing Comments and Adjournment

Dr. White said that the meeting would reconvene at 8:30 a.m. and briefed the attendees on meeting logistics. The meeting was adjourned at 4:28 p.m.

VI. Introductions, December 13

Dr. McMartin called the meeting to order at 8:35 a.m. and asked the attendees to introduce themselves (see Section II). Attending by teleconference on December 13 were Mary Schubauer-Berigan, Avima Ruder, and Kevin Dunn, of NIOSH (Cincinnati); Glinda Cooper, of EPA (Washington); and Jennifer Ratcliffe, of ILS. Dr. White briefed the attendees on conflict of interest guidelines and on meeting logistics.

VII. Draft RoC Monograph for Pentachlorophenol and By-products of Its Synthesis, Continued

VII.A. Draft Cancer Evaluation Component, Continued

VII.A.1 Cancer Studies in Humans, Continued

VII.A.1.1 Presentation on Issues Raised by the Panel

Drs. Lunn and Jahnke provided further information and clarification of issues raised in the December 12 peer-review comments and Panel discussion; the presentation reflected input from Dr. Schubauer-Berigan, as well as the RoC staff.

Dr. Lunn said that it is the opinion of the RoC staff that the epidemiological database does not allow the effects of pentachlorophenol to be separated from the effects of its by-products. Therefore, the relevant human exposure is exposure to a mixture of pentachlorophenol and its by-products. The by-products have dioxin-like activities, and the possibility cannot be ruled out that they are contributing any increased risks of cancer observed for exposure to this mixture.

The Panel was provided a list showing the chemical compositions of technical-grade pentachlorophenol products from several past manufacturers. Dr. Jahnke said the information was derived from the manufacturers' reports and published by the World Health Organization. The technical-grade products contained around 90% pentachlorophenol, plus di-, tri-, and tetra-chlorophenol, higher chlorinated phenoxyphenols, hexachlorobenzene, dioxin-like chemicals, and furans. The Panel also was provided with the results of analyses for impurities in the pentachlorophenol products used in the carcinogenicity studies in mice and rats. The list included the TCDD toxic equivalency factors for the impurities. The chemicals found in the technical-grade products used in the animal studies matched closely with the chemicals listed by the manufacturers. The by-products found were consistent with what would be expected from production of pentachlorophenol, and the composition varied from product to product.

Dr. Jahnke read a response from Mr. Dunn stating that there is no indication that any pentachlorophenol is imported into the United States other than from one source in Mexico, which does not use the hexachlorobenzene production method, and that no studies have been found on human exposure to pentachlorophenol produced by the hexachlorobenzene method.

Dr. Bandiera noted that the diphenylethers were the major impurities in the 90.4% technical-grade pentachlorophenol used in the animal studies, but were not included in the chemical compositions provided by manufacturers. Dr. Jahnke was unsure whether this meant that they were not detected or not measured.

Dr. Smith noted that for some impurities (such as heptachlorodibenzodioxin), the level differed greatly between the technical products used in the animal studies. He wondered whether it was reasonable to conclude that this extraordinary variation existed among the technical products used in the human studies. Dr. Smith said that even for heptachlorodibenzodioxin, the levels varied widely in the chemical compositions provided by the manufacturers. Therefore, it cannot be assumed that any group of humans in a particular location, job, or setting was meaningfully exposed to this by-product. Dr. Talaska said exposure to the by-products could vary even within a one-day period.

Mr. Dunn responded regarding the issue of consistency of human exposure to pentachlorophenol by-products, stating that for the plants in the pentachlorophenol producers cohort studies, samples had been analyzed for these constituents from 1940 through 1980. Although the levels varied depending on production conditions, such as heat or pressure, the constituents were present consistently throughout the history of pentachlorophenol production. The consistent presence of these constituents is a concern for exposure to pentachlorophenol products. The fact that these are consistent by-products of pentachlorophenol synthesis means that one cannot be exposed to a technical-grade pentachlorophenol product without being exposed to these constituents, which consistently amount to a TCDD toxic equivalent of greater than 1.

Dr. Lunn summarized the information from the three studies that evaluated exposure-response relationships between pentachlorophenol exposure and NHL. The Canadian sawmill workers cohort study found significant exposure-response relationships for both mortality and incidence and both lagged and unlagged exposure. The panelists were concerned that the relationship was not monotonic. In response to Dr. Smith's question regarding the plotting of the Friesen *et al.* (2007) data, in the modeling analysis, exposure was treated as a continuous (rather than categorical) variable, and the authors characterized the exposure-response relationship as "roughly monotonic." Dr. Lunn confirmed that in the presentation on December 12, the graph showed the modeled data, not the original data. Dr. Lunn commented that occupational exposure studies often find non-monotonic exposure-response relationships, possibly as a result of measurement error or a healthy worker survivor effect. Dr. Smith agreed that monotonic relationships should not necessarily be expected, but he emphasized that the use of the model data from Friesen *et al.,* rather than the original data, forced the relationship to appear monotonic, when it was not actually monotonic. Dr. Villeneuve agreed with Dr. Smith's comments and noted that Friesen *et al.* had assigned mean values to the five exposure categories and used these means (effectively one data point per exposure category) in their modeling, whereas the preferred approach would be to model the individual data.

Dr. Lunn noted that the NIOSH pentachlorophenol producers cohort study did not find an association between NHL and duration of employment. Duration of employment may not have been the best surrogate for exposure level, but no job exposure matrix was available for pentachlorophenol exposure. For this reason and because the number of cases was small, this lack of an exposure-response relationship is viewed as non-informative rather than evidence against an association. Dr. Schubauer-Berigan said a healthy worker survivor effect might have been a factor when exposure was based on duration of employment.

Dr. Lunn stated the Michigan pentachlorophenol producers cohort study found an exposure-response relationship between NHL and pentachlorophenol dioxin by-products; the risk was highest for the individuals in the highest exposure category. The response was not monotonic, but this could have been due to uncertainty of exposure classification; confidence in the classification was lower for low and medium exposure than for high exposure. Also, the analysis was limited by the small number of cases (8 cases). The earlier study of this cohort by Ramlow *et al.* also found an association with cumulative pentachlorophenol exposure; however, the disease codes combined NHL with multiple myeloma.

Dr. Lunn noted again that the external analysis in the Canadian sawmill workers cohort study used all sawmill workers, and was not specific for pentachlorophenol exposure. A healthy worker effect could also have influenced the external analysis. Therefore, the lack of an association with NHL in the external analysis is not considered to lessen the value of this study. Dr. Schubauer-Berigan added that the healthy worker effect is more of a problem in external analyses when dealing with a substance with relatively low potency.

Regarding potential confounding by TCDD exposure and whether the NIOSH study argues against an association between pentachlorophenol and NHL, Dr. Lunn explained that the Michigan pentachlorophenol producers cohort study included two subcohorts: a TCP-exposed cohort (of over 1,600) and a pentachlorophenol-exposed cohort (of about 770). The findings for the TCP-exposed cohort were reported in other publications. However, these populations overlapped by 196 workers who were exposed to both pentachlorophenol and TCP. These subcohorts were established primarily to look at the effects of dioxins (TCDD in the TCP cohort and higher chlorinated dioxins in the pentachlorophenol cohort). The job exposure matrix had been set up to classify workers as exposed to pentachlorophenol only, TCP only, or pentachlorophenol +TCP. In Collins *et al.* (2009), dioxins were measured in serum samples from representative subsets of these workers 20 years after they had been exposed. Primarily the higher chlorinated dioxins (the dioxin fingerprint) were seen in the pentachlorophenol workers, primarily TCDD was seen in the TCP workers, and both were seen in the pentachlorophenol +TCP workers. Thus, the serum profiling was consistent with the exposure assessment.

The biomonitoring data are also relevant to the NIOSH pentachlorophenol producers cohort study. Of the 675 workers classified in the NIOSH study as exposed to pentachlorophenol +TCP, no more than 196 would have had elevated TCDD levels (based on the serum profiling results of Collins *et al.,* 2009); the rest would have had the higher chlorinated dioxin profile. Furthermore, most (77%) of the workers classified as exposed to pentachlorophenol +TCP in the NIOSH study were from the Michigan plant. Therefore, the elevated SMR for workers exposed to pentachlorophenol +TCP was heavily influenced by the presence of workers from the Michigan plant. This lessens the argument for confounding by TCDD in the NIOSH study, as many of the workers classified in the NIOSH study as exposed to pentachlorophenol +TCP would not have had elevated serum TCDD levels. Therefore, the NIOSH study does not contradict the findings of Collins *et al.* (2009). Dr. Ruder concurred with Dr. Lunn's interpretation and said that much of the difference in the exposure classification between the studies was probably due to workers who were not exposed heavily to TCP.

Dr. Lunn stated that the Canadian sawmill workers cohort study is considered to provide strong evidence of an association between pentachlorophenol exposure and NHL, based on the large number of cases; analysis of incidence; a strong exposure-response relationship, even if it is not monotonic, that is consistent for both incidence and mortality; and lack of evidence for confounding. The Michigan pentachlorophenol producers cohort study is considered to provide supporting evidence based on an association between pentachlorophenol exposure and NHL in the external analysis (about a twofold increase in risk); an association with cumulative exposure, though not specific for NHL; and observation of the highest risk in the workers with highest blood levels of pentachlorophenol by-products (though there was no trend analysis). The power of the analysis is limited by the small number of cases. Concerning potential confounding by TCDD, Dr. Lunn noted that separate analysis of the cohort of TCP-exposed workers found little evidence for an association between TCDD and NHL. The NIOSH pentachlorophenol producers cohort study found a stronger association between NHL and exposure to pentachlorophenol +TCP than for exposure to pentachlorophenol alone; however, this result may have been influenced by the TCP exposure classification issue previously discussed. This study had a larger number of cases than the Michigan pentachlorophenol producers cohort study. No association was seen between NHL and exposure duration. Although confounding by TCDD is considered unlikely, confounding by other agents is possible. In the nested case-control study, the three NHL deaths occurred in the people with the highest pentachlorophenol exposure, but the sample size was too small to detect a statistically significant association. Little information was available on potential TCDD exposure; the three deaths all occurred in a cohort of workers that apparently was not exposed to herbicides other than pentachlorophenol, but their exposure to other chemicals was not known.

VII.A.1.2 Panel Discussion, Continued

Dr. Villeneuve noted that the Canadian sawmill workers cohort study found a borderline positive association between TeCP and NHL; he wondered whether there was any correlation between pentachlorophenol and TeCP exposure in that study, and thus potential confounding. Dr. Zahm said the correlation was 0.45. Dr. Lunn noted that the analysis by Friesen *et al.* indicated that the more specific the analysis was for pentachlorophenol exposure, the stronger the association with NHL. Dr. Villeneuve would have liked to see whether co-exposure to TeCP changed the risk estimate for pentachlorophenol.

Dr. Ward said the large numbers of cases in the Canadian sawmill workers cohort study was striking and was not emphasized in the monograph. Using the number of exposed combined in the three higher pentachlorophenol exposure groups (exposure > 1 year), she roughly estimated SIRs for ever exposed (versus never or low) to pentachlorophenol of 1.8 for NHL and around 2 for multiple myeloma, which would most likely be highly significant; however, she noted that the denominators were not provided to calculate the actual risk estimates. She found it difficult to know how much importance to attach to exposure duration, because there is no good sense of the intensity of exposure, and looking only at duration could result in serious misclassification of exposure. Dr. Ward said she was moving towards a more positive interpretation of the study, which is the most informative study, in a large population, with clearly intense dermal exposure.

Dr. Smith agreed that adjustment for the healthy worker effect could be very important in studies with small relative risks. However, adjustment for a healthy worker effect would not alter the exposure-response findings in these pentachlorophenol studies. He noted that in the NIOSH pentachlorophenol producers cohort study, a test for trend on the exposure-duration results would probably show a statistically significant decrease in SMR from the low-exposure group to the high-exposure group. He did not see how these results could be used as evidence in support of an increased risk. He agreed that the Canadian sawmill workers cohort study was the strongest study; however, he would not call the exposure-response relationship strong, given that risk went down with increased exposure and then up.

Dr. McMartin asked that the four primary reviewers share their thoughts on the evidence based on having given it further thought and after hearing the morning's presentation.

Beyond the remarks he had just made, Dr. Smith said that he found the data on the variation in pentachlorophenol contaminant levels to be exceedingly important. He said it was not sufficient to characterize by-product levels in just one cohort. If the levels of pentachlorophenol by-products vary considerably in the commercial products, then he would not recommend combining them in the listing, which to him implies that they are constant. He noted that if a chemical were proposed to be carcinogenic with its by-products, without differentiating between the chemical and its by-products, then there would be no opportunity for industry to clean up the production process to reduce specific carcinogenic by-products. Also, if the by-products are important to carcinogenicity, then epidemiological evidence cannot be synthesized across different study populations, because their exposure to by-products will differ.

Dr. Smith said he both disagreed with the idea of listing pentachlorophenol and by-products of its synthesis and considered the issue of variability in the by-products to be a limitation of the epidemiology data. Dr. Sandy commented that the scientific community has previously drawn conclusions about the carcinogenicity of even very diverse complex mixtures; most recently, IARC has identified air pollution as a carcinogen. She noted that despite the variability in the by-products of pentachlorophenol, there are commonalities. She asked whether Dr. Smith was uncomfortable with making conclusions about such variable mixtures in general. Dr. Smith said that it was not the idea of mixtures *per se,* but the very wide range of variation in the by-product levels (i.e., up to 100-fold) that made him wonder about the epidemiological evidence for pentachlorophenol. Dr. Talaska agreed with Dr. Sandy and noted that this level of day-to-day variability is not unusual for complex mixtures. For almost any complex mixture of polycyclic aromatic hydrocarbons, including perhaps tobacco smoke and certainly coal-tar pitch, one would see at least this much variability. He said that if industry were able to change the production methods to reduce the contaminants, then the carcinogenicity of the substance could be re-studied.

Dr. Nesnow agreed that a lot of analysis has been done on complex mixtures; however, the major difference is that decisions about their carcinogenicity to humans were based on strong mechanistic evidence; many of the components were strong genotoxins. That is not the case with pentachlorophenol: there is one major epidemiological study that shows a mild effect and small exposure-response relationship; the mechanistic data are not helpful; and the animal data are not consistent. Basically, the decision comes down to the strength of the evidence from human cancer studies.

Dr. Beane Freeman agreed that the Canadian sawmill workers cohort study was clearly the strongest study, but noted that the exposure-response relationship was based on duration of exposure, with no information on intensity of exposure. She found the other studies to be less informative and somewhat supportive of a relationship between pentachlorophenol and NHL. However, she did not view the whole body of epidemiological evidence as supporting the listing of pentachlorophenol as a known human carcinogen, for many of the reasons previously discussed. Dr. Zahm said she would support the conclusion that there was limited evidence of carcinogenicity from the human cancer studies. Dr. Villeneuve said that there was little evidence to support exposure-response relationships, and that he agreed that the evidence for carcinogenicity from the human cancer studies was limited.

VII.A.1.3 Action

The Panel recommended changing the NTP's level of evidence conclusion in the draft monograph from *sufficient* to *limited* evidence of carcinogenicity of pentachlorophenol and by-products of its synthesis from studies in humans. Dr. Villeneuve moved to accept the amended conclusion. Dr. Smith seconded the motion.

The Panel agreed unanimously (10 yes, 0 no, 0 abstentions) that the scientific information presented from human cancer studies supports the level of evidence conclusion of *limited evidence of carcinogenicity* of pentachlorophenol and by-products of its synthesis. The basis is limited evidence across studies in humans, which indicates a causal association between pentachlorophenol and by-products of its synthesis and non-Hodgkin lymphoma was credible, but alternative explanations such as chance, bias, or confounding factors could not be adequately excluded.

VII.A.2 Studies in Experimental Animals

VII.A.2.1 Presentation

Dr. Jahnke presented an overview of the key information in the draft monograph section on studies in experimental animals. The studies identified in the literature review included chronic cancer studies, studies of pentachlorophenol as a co-carcinogen and as an enzyme inhibitor, and short-term gene knockout and transgene studies. The test substances included technical-grade pentachlorophenol, Dowicide EC-7, and analytical-grade pentachlorophenol (99% pure). All of the studies in experimental animals were considered adequate for study-quality evaluation. The most informative studies in both rats and mice were NTP studies that included sufficient numbers of animals, full chemical characterization, dose selection to induce mild toxicity, sufficient study duration, and complete histopathology and reporting.

The most informative studies in rats were a two-year study in F344 rats of both sexes and a two-year stop-exposure study with one year of exposure in male F344 rats, both of which used 99% pure pentachlorophenol. In the stop-exposure study in male rats, the incidence of malignant mesothelioma was significantly increased, and a non-significant increase in the incidence of squamous-cell carcinoma of the nose was considered to be exposure related because it exceeded the historical control range. Other studies in rats were limited by inadequate numbers of animals, pathology, reporting, and chemical characterization of the test

material. A cancer study with Dowicide EC-7 found no increase in total tumor incidence, and a co-carcinogen study with technical-grade pentachlorophenol found an increased incidence of hepatoma in female rats that could have been due to contamination with TCDD or tetrachlorodibenzofuran.

The most informative studies in mice were two-year studies of exposure to technical-grade pentachlorophenol and Dowicide EC-7 in B6C3F_1 mice of both sexes. Significant dose-related increases in the combined incidence of benign and malignant hepatocellular tumors were observed in male mice exposed to technical-grade pentachlorophenol and mice of both sexes exposed to Dowicide EC-7. Some separate incidences of benign and malignant hepatocellular tumors also were significantly increased. In mice exposed to Dowicide EC-7, significant dose-related increases were observed in the combined incidences of benign and malignant pheochromocytoma (tumors of the adrenal medulla) in both sexes and hemangioma and hemangiosarcoma (blood-vessel tumors) in females. In mice exposed to technical-grade pentachlorophenol, significant dose-related increases were observed in the incidences of benign pheochromocytoma in males and malignant hemangioma and hemangiosarcoma in females.

Other studies in mice were limited by short duration, small numbers of animals, and limited histopathological examination. No exposure-related tumors were observed in a cancer study with Dowicide EC-7, two mechanistic studies with 99% pure pentachlorophenol, or a cancer screening study in *p53*(+/–) mice fed 99% pure pentachlorophenol. A study of Tg.AC (v-*ras*^{Ha}) mice exposed dermally to 99% pure pentachlorophenol found significantly increased incidences of dermal papilloma; however, the Tg.AC model has a problem with false-positive results, so the relevance of the reporter gene activation mechanism underlying this model to mechanisms of human cancer is unclear.

The NTP concluded that there is sufficient evidence of carcinogenicity in experimental animals based on significant increases in malignant mesothelioma of the *tunica vaginalis* in male rats, non-significantly increased incidence greater than historical control incidences of rare nasal-cavity squamous-cell carcinoma in male rats, and significant increases in liver and adrenal-gland tumors in mice of both sexes and blood-vessel tumors in female mice.

Dr. Nesnow asked for Dr. Jahnke's reaction to the statement in the public comments that 99% pure pentachlorophenol was not carcinogenic in rats because no tumors were observed in the two-year chronic exposure study. Dr. Jahnke noted that tumors were found in rats exposed to a higher dose of 99% pure pentachlorophenol for a shorter duration (in the stop-exposure study). Dr. Selgrade asked whether the difference was due to the higher dose in the stop-exposure study, and whether the higher dose resulted in toxicity. Dr. Jahnke said the NTP report considered the stop-exposure study to provide "some" evidence for carcinogenicity in male rats. Dr. Bucher said that since pentachlorophenol exposure causes initial weight loss, which suppresses tumor responses, it could be speculated that the stop-exposure study allowed the animals to recover from that effect. However, no experimental evidence is available to support any speculative explanations for the difference in the results of the two studies.

In response to questions from Drs. Talaska and Smith, Dr. Jahnke said the NTP studies of TCDD exposure in mice found liver tumors, primarily hepatoma, but also hepatocellular

carcinoma. Dr. Nigel Walker added that effects in rats were primarily liver and lung tumors, but not malignant mesothelioma. Dr. Jahnke said the section of the monograph on mechanistic studies looks at liver tumors in mice with respect to the relative contributions of dioxin toxic equivalents and other by-products in the technical-grade pentachlorophenol and Dowicide EC-7. This analysis indicates that dioxin-like factors were not the only contributors to liver carcinogenicity, and that a component corresponding to pentachlorophenol alone also contributed. Dr. McMartin noted that the mouse studies with technical-grade pentachlorophenol and Dowicide EC-7 gave very similar results, despite the differences in the by-products they contained. Dr. Nesnow asked about a reference in the mechanistic section to liver tumors in female Wistar rats. Dr. Jahnke said these were the hepatomas observed in the study where a small amount of TCDD was present.

VII.A.2.2 Peer Review Comments and Panel Discussion

Dr. Sandy, first reviewer, found the overall approach for preparing the cancer assessment of the experimental animal studies to be appropriate. She agreed that the NTP studies were the most informative. In the rat studies with 99% pure pentachlorophenol, observation of the rare nasal squamous-cell carcinoma at incidences higher than in the historical control not only in the stop-exposure study, but also in the lower-dose males in the two-year study tends to suggest that something may be going on in the nose of the rat. The stop-exposure study also found a statistically significant increase in the incidence of malignant mesothelioma in the abdominal cavity of male rats.

Dr. Sandy said in the NTP studies of male mice, significant dose-related increases were observed in the incidences of liver carcinoma and of liver adenoma and carcinoma combined with both technical-grade pentachlorophenol and Dowicide EC-7. A significant dose-related increase in benign adrenal medulla tumors was seen in male mice exposed to technical-grade pentachlorophenol and a significant dose-related increased in benign and malignant adrenal medulla tumors in male mice exposed to Dowicide EC-7. Thus, the results of the studies with these two formulations seem to be consistent. In the female mice, significant dose-related increases in hemangiosarcoma were observed with both technical-grade pentachlorophenol and Dowicide EC-7; again, the findings are consistent. The female mice exposed to Dowicide EC-7 also showed significant dose-related increases in the incidence of liver adenoma and carcinoma combined and of benign and malignant adrenal medulla tumors.

Thus, multiple studies gave positive results in mice of both sexes and for multiple tumor sites, and studies in male rats found rare nasal squamous-cell carcinomas and a significant increase in abdominal-cavity mesothelioma, probably originating from the *tunica vaginalis*.

Dr. Selgrade wondered whether the nasal-cavity carcinoma could have been due to inhalation exposure from the food; she noted that inhalation exposure was a potential route of occupational exposure. Dr. Talaska said the vapor pressure of pentachlorophenol was very low. Dr. Sandy said nasal tumors had been observed in other NTP dietary-exposure studies; active metabolites could be transferred to the nose via the circulation. Dr. McMartin asked the Panel to address the public comment that the mesothelioma of the *tunica vaginalis* observed in male rats was specific to male F344 rats and was therefore not relevant to human cancer. Dr. Sandy said that this tumor type is highly invasive and, as far as she is aware, was considered to

be a relevant indicator of human carcinogenicity. Dr. McMartin asked the Panel to respond to public comments that the by-products of pentachlorophenol synthesis were not carcinogenic and that the carcinogenicity of technical-grade pentachlorophenol in the animal studies should not be given weight in the evaluation. Dr. Talaska noted that the charge to the Panel was to consider the potential carcinogenicity of products of pentachlorophenol synthesis, as well as pentachlorophenol itself.

VII.A.2.3 Action

Dr. Nesnow moved to accept the NTP's preliminary recommendation regarding the evidence of carcinogenicity in experimental animals. Dr. Sandy seconded the motion. The Panel agreed unanimously (10 yes, 0 no, 0 abstentions) that the scientific information presented from studies in experimental animals supports the NTP's level of evidence conclusion of sufficient evidence of carcinogenicity of pentachlorophenol and by-products of its synthesis based on increased incidence of tumors in rats and mice at several tissue sites.

VII.A.3 Disposition, Toxicokinetics, and Mechanistic and Other Relevant Data

VII.A.3.1 Presentation

Dr. Jahnke presented an overview of the key information in the draft monograph sections on disposition, toxicokinetics, and mechanistic data and described the scope of literature on pentachlorophenol metabolism and mechanistic studies.

Pentachlorophenol is efficiently absorbed via dermal, oral, or inhalation exposure; it is widely distributed and can accumulate in tissues, but accumulation is limited by extensive binding to plasma proteins. Pentachlorophenol is excreted primarily in the urine; clearance is slower and the excretion half-life is longer in humans than in rats.

The genotoxicity of pentachlorophenol is mediated by its metabolites. Pentachlorophenol has been shown to induce DNA damage in cells with metabolic capability and in metabolically incompetent cells with exogenous metabolic activation. There is evidence of chromosomal damage and apoptosis in cells with metabolic capability, evidence of formation of C8-dG O-adducts in rodent liver, and limited evidence for chromosomal aberrations in exposed workers. The pentachlorophenol metabolite tetrachlorohydroquinone (TCHQ) itself is mutagenic.

Although the mechanisms of NHL are not known, NHL is strongly associated with immunosuppression. Pentachlorophenol exposure also has been linked to immunosuppression: apoptosis has been observed *in vitro* in human lymphocytes and Jurkat T cells exposed to pure pentachlorophenol; people exposed to pentachlorophenol-containing pesticides show suppression of cellular and humoral immunity; and both pure and technical-grade pentachlorophenol are immunosuppressive to human lymphocytes affecting both cell-mediated and humoral immunity. Immunosuppression by dioxins is believed to be mediated by activation of the aryl hydrocarbon receptor, which affects numerous downstream biochemical pathways.

Dr. Jahnke said the mechanistic data demonstrate a level of biological plausibility for the carcinogenicity of pentachlorophenol in humans. Proposed modes of action include metabolism to genotoxic and mutagenic metabolites, DNA damage (e.g., strand breaks), DNA adduct

formation, chromosomal aberrations and breakage, immunosuppression, and inhibition of apoptosis. Pentachlorophenol exposure causes biological effects similar to those seen in NHL, including immunosuppression and DNA damage, chromosome breakage, and inhibition of apoptosis.

Dr. Nesnow recommended some corrections to the monograph. C8-dG O-adducts were seen only in calf thymus DNA, and not *in vivo* or in cells. The adducts formed *in vivo* were essentially 8-oxodG adducts. He said in the monograph, Figure 5-2 is incorrect, and Figure 5-1 appears to imply that there were adducts to bases other than dG.

VII.A.3.2 Peer Review Comments and Panel Discussion

Dr. Bandiera, first reviewer, found the information presented in the monograph and in the presentation to be very good. He suggested including more information about the half-life of pentachlorophenol in humans; the monograph cites two contradictory studies, one indicating a relatively short half-life. He identified information for inhalation exposure indicating that the half-life is more like 16 days. He said the monograph should emphasize that the half-life of pentachlorophenol in humans is relatively long. The studies describing the possible metabolites formed were plausible. Dr. Bandiera noted that cytochrome P450 also has peroxidase activity and could possibly produce TCHQ in various tissues.

Dr. Selgrade, second reviewer, commented that the preponderance of evidence suggests that the immunosuppressive effects of pentachlorophenol are due to the contaminants. In *in vivo* animal studies, effects were seen with technical-grade but not analytical-grade pentachlorophenol. The one exception was the Kerkvliet study (Kerkvliet *et al.,* 1982). In the animal *in vitro* studies, the results were not very impressive, and in the human lymphocyte studies, the purity of the pentachlorophenol was not reported. She said these results are not surprising, given that dioxins are very immunosuppressive.

Dr. Nesnow, third reviewer, concurred with the previous reviewers. He found the section to be well written, but provided a few corrections. He suggested the section on DNA adduct formation be rewritten to accurately reflect Dai *et al.* (2005). He said the statement that pentachlorophenol was mutagenic in human lymphocytes is incorrect. Dr. Nesnow agreed to provide a new reference dealing with Nrf2 [nuclear factor erythroid 2-related factor 2], which is consistent with oxidative stress. He considered very important and relevant the information on the production in rodents of the quinone and hydroquinone metabolites and TCP, which bind to proteins and have the potential to bind to DNA.

Dr. Talaska said the discussion of metabolism and elimination of conjugates needed to be consistent in the monograph. Dr. McMartin asked the panel to address two issues raised in the public comments: (1) that the overwhelmingly negative results of the *in vivo* genotoxicity assays made it implausible that mutation plays a role in pentachlorophenol carcinogenicity and (2) that the monograph did not address high-dose pentachlorophenol cytotoxicity-driven mechanism-of-action events, such as oxidative-stress-induced DNA damage and chronic inflammation. Dr. Bandiera agreed that oxidative stress was not well addressed in the monograph and that more could be said about ROS and inflammation as a possible preliminary step towards carcinogenicity. Dr. Nesnow did not agree that ROS formation is a high-dose issue; formation

of the metabolites involved in redox cycling is not related to dose. As for the *in vivo* rodent studies, there is little evidence for genotoxicity, but two studies do show positive results.

VII.A.4 Overall Cancer Evaluation

VII.A.4.1 Presentation

Dr. Jahnke presented an overview of the overall cancer evaluation. Studies in humans demonstrate a causal relationship between exposure to pentachlorophenol and by-products of its synthesis and NHL. The biological plausibility of a multi-site carcinogen is supported by findings of multiple cancer sites in studies of rats and mice with chronic dietary exposure and mechanistic data demonstrating biological plausibility in humans. Little is known about mechanisms of NHL in humans, but pentachlorophenol exposure data suggest overlap of mechanisms and associations that occur with NHL: immunosuppression and DNA damage (strand breaks), chromosome breakage, and inhibition of apoptosis.

The NTP's preliminary listing recommendation is that pentachlorophenol and by-products of its synthesis is *known to be a human carcinogen* based on sufficient evidence from studies in humans demonstrating a causal relationship between exposure to pentachlorophenol and non-Hodgkin lymphoma. This conclusion is supported by sufficient evidence in experimental animals and supporting mechanistic evidence.

Dr. McMartin suggested revising the listing recommendation based on the Panel's conclusions concerning the human cancer studies.

VII.A.4.2 Peer Review Comments and Panel Discussion

Dr. Nesnow, first reviewer, commented that although there was some evidence for an association with multiple myeloma, the review had addressed primarily the evidence for an association with NHL, and evidence for cancer in humans was found to be limited. He said the statement that pentachlorophenol is a multi-site carcinogen in humans should be revised or removed. Overall, Dr. Nesnow found the section to be well written and comprehensive, integrating the human cancer data, experimental data, and mechanistic data. However, it is not as broad as it could be, because it focuses only on NHL. He suggested adding more discussion of the data on liver cancer and its mechanisms, including the data on reactive metabolites in rat liver. Also, pentachlorophenol's ability to deplete glutathione, as well as inducing ROS, should be mentioned. Because pentachlorophenol causes a broad spectrum of tumor types in animals, there should be more discussion of the different types of damage measured in rodents. He agreed with the overall conclusion about the mechanistic data.

Dr. Villeneuve, second reviewer, said he expected the RoC staff to revise the preliminary paragraphs to be consistent with the panel's discussion of the key issues. He suggested mentioning the weak support for an exposure-response relationship with NHL, the small sizes of the relative risks, and the relatively small number of human cancer studies.

Dr. Sandy, third reviewer, suggested revising the fourth sentence of paragraph 3 on page 125 to say, "dietary exposure to pentachlorophenol caused tumors at multiple tissue sites in *male* rats and *mice of both sexes."* She also suggested revising the third sentence of paragraph 1 on

page 126 to say, "pentachlorophenol caused *malignant or a combination of malignant and benign* liver tumors in *male and female* mice . . ."

Dr. Beane Freeman, fourth reviewer, suggested finding a better reference for the first sentence in the section on immunosuppression on page 127. She also noted, with respect to the statement on page 128 that dioxin has been linked to NHL in humans, that IARC's determination on dioxin was based on all cancers, not NHL specifically. Dr. Lunn noted that IARC had found limited evidence for an association between dioxin and NHL.

Dr. Selgrade recommended the statement, "there is sufficient evidence that pentachlorophenol itself is immunosuppressive," should be removed, because the preponderance of the data for rodents is for technical-grade pentachlorophenol.

Dr. Smith reiterated his discomfort with the combined listing of pentachlorophenol and by-products of its synthesis. He stated that the animal evidence, including the comparison between technical grade pentachlorophenol and Dowicide EC-7, supports the carcinogenicity of pentachlorophenol, and not its by-products, and that there is no good animal or human evidence that the pentachlorophenol by-products were responsible for the carcinogenic effects. He preferred to vote on pentachlorophenol alone as *reasonably anticipated to be a human carcinogen*. This was based in part on consideration of the precedent that this combined listing would set for the future. Dr. Talaska said it would be difficult to demonstrate the carcinogenicity of pentachlorophenol alone from the available human studies. Dr. Smith did not see that as a hindrance to listing pentachlorophenol as *reasonably anticipated to be a human carcinogen*. Dr. Nesnow suggested that the listing specifically state that the evidence from some studies in experimental animals is for the carcinogenicity of pure pentachlorophenol. Dr. Sandy asked whether the panel could make a recommendation that there was sufficient evidence of carcinogenicity of pentachlorophenol itself from studies in experimental animals. Dr. Bucher noted that these issues had been considered in the decision to recommend the combined listing. The main consideration was that the most informative studies in mice used only technical-grade material, whereas the studies in rats used only pure pentachlorophenol, making it difficult to meet the criterion of evidence from two different animal species for pentachlorophenol alone. Also, there were no human cancers studies of pure pentachlorophenol.

Noting that the rat studies with pure pentachlorophenol, causing rare nasal tumors, and the mice studies with Dowicide EC-7 in fact provided evidence for the carcinogenicity of pentachlorophenol alone. Dr. Sandy moved that the panel vote on the question of whether there was sufficient evidence of carcinogenicity of pure pentachlorophenol from studies in experimental animals in multiple species and multiple sites. The motion was seconded by Dr. Nesnow.

Dr. Lunn noted that explanation would be needed of exactly how the criteria were met, including explanation of how the studies in mice with Dowicide EC-7 support the carcinogenicity of pure pentachlorophenol. Dr. Selgrade questioned whether there was enough evidence to support the carcinogenicity of pure pentachlorophenol in rats; the increased incidence of the rare nasal cavity tumors was not statistically significant, and the only significantly increased tumor incidence was for malignant mesothelioma in the high-dose stop-exposure study.

Dr. Smith said technical grade pentachlorophenol and Dowicide EC-7 have different contaminant patterns; technical-grade pentachlorophenol contains various dioxin-like contaminants, whereas Dowicide EC-7 contains primarily TeCP, which is not a carcinogen. This provides evidence that pentachlorophenol itself is responsible for the carcinogenicity in the mice studies.

Dr. Lunn noted that Appendix F provided information on the dioxin toxic equivalents to which mice were exposed from technical-grade pentachlorophenol and Dowicide EC-7 (using the worst-case scenario). Dr. Jahnke said the total dioxin toxic equivalents to which the mice were exposed were much greater for technical-grade pentachlorophenol than in Dowicide EC-7. The monograph concludes that the dioxin-like by-products apparently contributed to liver tumor formation, based on the higher incidence of liver tumors in mice exposed to technical-grade pentachlorophenol than in mice exposed to Dowicide EC-7, and technical-grade pentachlorophenol contained dioxin toxic equivalent levels within the range of TCDD levels associated with liver carcinogenicity. Dr. Sandy noted that the monograph stated that exposure to Dowicide EC-7 (with low dioxin toxic equivalents) resulted in increased liver tumor formation, suggesting that pentachlorophenol, possibly in concert with other by-products, contributed to the liver tumor response. Dr. Smith said the issue of whether pentachlorophenol by-products may have contributed to carcinogenicity in some studies is separate from the issue of whether pentachlorophenol alone is carcinogenic in animals, for which the evidence is overwhelming.

Dr. McMartin reminded the Panel that they had voted earlier that the scientific information presented from studies in experimental animals supports the conclusion of sufficient evidence of carcinogenicity of pentachlorophenol and by-products of its synthesis and suggested reviewing the presentation slides listing the tumor sites that supported the call. Dr. Sandy stated that all the tumors sites (malignant mesothelioma and rare nasal cavity squamous-cell carcinoma in male rats, and liver tumor, adrenal gland tumors and blood vessels in mice) observed for pentachlorophenol and byproducts of its synthesis (based on studies of pure pentachlorophenol, technical grade pentachlorophenol, Dowicide EC-7) would also apply to pentachlorophenol by itself (based on studies of pure pentachlorophenol, and Dowicide EC-7) except that tumors in blood vessels were only seen in one sex in mice (Dowicide EC-7) rather than both sexes (technical grade pentachlorophenol). Dr. McMartin stated that the findings in rats using pure pentachlorophenol were only relevant to pentachlorophenol itself and not pentachlorophenol and its byproducts. He suggested relying on the RoC staff to determine which tumor sites were relevant for pentachlorophenol itself and pentachlorophenol and byproducts of its synthesis.

VII.A.4.3 Action

Dr. McMartin asked the panel to vote on a revised evaluation of the evidence for carcinogenicity in experimental animals. Dr. Sandy moved that the Panel approve the revised conclusion: There is sufficient evidence of the carcinogenicity of pentachlorophenol and of the carcinogenicity of pentachlorophenol and by-products of its synthesis from studies in experimental animals. Dr. Talaska seconded the motion. The panel agreed unanimously (10 yes, 0 no, 0 abstentions).

VII.A.4.4 Action

Dr. McMartin asked the panel to vote on the revised listing recommendation that pentachlorophenol and by-products of its synthesis is *reasonably anticipated to be a human carcinogen* based on limited evidence from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors could not be reasonably be excluded; and on sufficient evidence of carcinogenicity of pentachlorophenol and of pentachlorophenol and by-products of its synthesis from studies in experimental animals and supporting mechanistic evidence. Dr. Smith moved to approve the revised listing recommendation; Dr. Sandy seconded the motion, and the Panel agreed unanimously (10 yes, 0 no, 0 abstentions).

VII.B. Draft RoC Substance Profile

Dr. Jahnke summarized the contents of the draft substance profile as containing the preliminary listing status recommendation, summarizing the scientific information key to reaching a recommendation, and providing information on properties, use, production, exposure, and existing federal regulations and guidelines.

Dr. McMartin asked that the reviewers address the issues in terms of the study descriptions in the profile that would be impacted by the Panel's recommendations, and suggest any additional studies that should be cited. Dr. Talaska, first reviewer, said no changes were needed relative to production and use, but that discussion of the current NHANES data should be added, including that exposure was higher in children than in adults. Dr. Smith, second reviewer, said the changes needed in the human cancer studies section should be clear from the discussion already on record. He said the focus should be on the four cohort studies, and that the case-control studies did not contribute to the evaluation. Dr. Sandy said the section on experimental animal studies should include information on the levels of contaminants in technical-grade pentachlorophenol and Dowicide EC-1, perhaps including the estimated worst-case dioxin toxic equivalents. Dr. Selgrade, third reviewer, said the information on immunosuppression and on DNA adducts in the section on mechanistic studies should be corrected, as discussed previously. Dr. Nesnow suggested that where the profile states that human liver microsomes have been shown to metabolize pentachlorophenol to TCHQ, references from the monograph should be added mentioning studies with homogenates and the work of Mehmood *et al.* (1996).

VIII. Closing Remarks on Draft RoC Monographs

Dr. McMartin thanked the Panel for their participation and recommendations. Drs. Jahnke and Lunn acknowledged and thanked those who contributed to putting together the draft monographs. Dr. Bucher thanked RoC staff for their work overnight on providing additional information. He also thanked the chair and the Panel for their remarkable effort in providing one of the more detailed and careful reviews that he had seen from any NTP expert panel.

The meeting was adjourned at 12:22 PM.

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Dr. Kenneth McMartin
Chair, Peer Review Panel

Date: _____