National Toxicology Program Response to the Peer-Review Report

Peer Review of the Draft Report on Carcinogens Monographs on *ortho*-Toluidine and Pentachlorophenol and By-products of its Synthesis

Public Meeting
December 12–13, 2013

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Introduction

The NTP convened an *ad hoc* scientific panel ("Panel") to peer review the draft Report on Carcinogens (RoC) monographs on *ortho*-toluidine and pentachlorophenol and by-products of its synthesis at a public meeting held December 12–13, 2013, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC (information on the meeting is available at http://ntp.niehs.nih.gov/go/38854). A draft RoC monograph consists of a cancer evaluation component and a substance profile. For each draft RoC monograph, the Peer-Review Panel had a two-fold charge:

1. To comment on the draft cancer evaluation component, specifically, whether it was 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 RoC listing criteria,

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

The Panel was asked to vote on each of the following for *ortho*-toluidine and pentachlorophenol and by-products of its synthesis:

1. Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from human cancer studies of the substance.

2. Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from experimental animal studies of the substance.

3. Whether the scientific evidence supports the NTP's preliminary listing decision for the substance in the RoC.

Per the process for preparation of the RoC, the NTP prepares a response to the peer review and posts it on the RoC website (http://ntp.niehs.nih.gov/go/38854). The *NTP Response to the Report on the Peer Review of the Draft Report on Carcinogens Monographs on* ortho-*Toluidine and Pentachlorophenol and By-products of its Synthesis* ("Peer-Review Report") includes NTP's response to the Panel's recommendations and scientific and technical peer-review comments.

The NTP carefully reviewed and considered the Peer-Review Report in revising the draft monographs. The revised draft RoC monographs[1] will be shared with the public and the NTP Board of Scientific Counselors (BSC) at their public meeting on April 17, 2014, and finalized following the meeting.

[1] Available at http://ntp.niehs.nih.gov/go/37898 [*ortho*-toluidine] and http://ntp.niehs.nih.gov/go/37897 [pentachlorophenol and by-products of its synthesis].

ortho-Toluidine and Pentachlorophenol and By-products of its Synthesis Peer-Review Panel[2]

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

ortho-Toluidine

The Draft RoC Monograph on *ortho*-toluidine was peer reviewed at a public meeting held December 12–13, 2013, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. The NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions, and (2) scientific and technical peer-review comments related to scientific issues and to improve the technical accuracy, clarity, and objectivity of the monograph. The Panel also provided several editorial comments, which are not included in the NTP response to the Peer-Review Report. These comments were also carefully considered in preparing the revised draft RoC monograph for *ortho*-toluidine.

Panel Recommendations and NTP Response

Panel Recommendations

The NTP's conclusion regarding U.S. exposure

The Panel agreed that a significant number of people in the United States are exposed to *ortho*-toluidine.

The NTP's preliminary listing decision for ortho*-toluidine in the RoC*

The Panel agreed unanimously (11 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list *ortho*-toluidine in the RoC as *known to be a human carcinogen* based on increased risks of urinary bladder cancer among exposed workers, in concert with cancer studies in animals, including site concordance with urinary bladder cancer in female and male rats and humans, and mechanistic data supporting biological plausibility in humans.

The NTP's conclusion regarding the level of evidence for carcinogenicity from human cancer studies

The Panel agreed unanimously (11 yes, 0 no, 0 abstentions) that the scientific information presented from human cancer studies supports the NTP's level of evidence conclusion of *sufficient evidence of carcinogenicity.* These studies found an increased risk of urinary bladder cancer among *ortho*-toluidine workers that is unlikely to be explained by chance, bias, or confounding.

The NTP's conclusion regarding the level of evidence for carcinogenicity from studies in experimental animals

The Panel agreed unanimously (11 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.* This conclusion was based on increased incidences of malignant tumors and combined malignant and benign tumors in two species and at several tissue sites: urinary bladder, connective tissue, subcutaneous tissue, mesothelium, blood vessel, or liver.

Scientific basis for sufficient evidence from human cancer studies, experimental

animal studies, and mechanistic data

NTP Response: The NTP concurs with the Panel that *ortho*-toluidine should be listed in the RoC as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies showing that it causes urinary bladder cancer in humans, together with studies showing that it causes urinary bladder cancer in rats, and studies demonstrating the biological plausibility of mechanisms of its carcinogenicity in humans.

Scientific and Technical Peer-Review Comments on the Draft RoC Monograph

The Panel provided comments on scientific issues regarding the Draft RoC Monograph on ortho-Toluidine and that would add to its clarity and completeness. The specific comments and NTP response to those comments are discussed below.

Comments and NTP's response related to scientific issues in the RoC monograph

Panel Comments:

- One Panel member suggested that the Canadian population case-control study on urinary bladder cancer (Richardson *et al.* 2007)[4] should not be included in the cancer assessment because of concerns about the exposure assessment and low exposure prevalence of *ortho*-toluidine among the study participants.

NTP Response: This study is identified as a lower quality study in the draft monograph, primarily because of the quality of the exposure assessment. The NTP agrees with the comments by the Panel and has clarified in the revised monograph the discussion of the study's limitations, specifically noting the low exposure prevalence. In general, the NTP's approach for including studies in its evaluation leans towards being inclusive, noting study limitations, and whether those limitations would generally lead to an underestimate or overestimate of the risk estimate, or the direction of the bias is not known. Thus, the study remains in the cancer evaluation in the revised draft monograph.

- One Panel member stated that the study on *ortho*-toluidine DNA releasing adducts found in urinary bladder tissue and urinary bladder tumors from humans (Böhm *et al.* (2011)[5] should not be cited in the monograph because the method used was not validated with synthetic adducts; however, other Panel members recommended including the study and noting the methodological limitations.

NTP Response: The monograph was revised to note the methodological limitations of the study. The study is cited in both the exposure and mechanistic sections of the document. The NTP believes the methodological limitations do not limit its utility as a marker of exposure because the method (acid hydrolysis) has been validated for many other tobacco-specific nitrosamines, although NTP acknowledges that the

[4] Richardson K, Band PR, Astrakianakis G, Le ND. 2007. Male bladder cancer risk and occupational exposure according to a job-exposure matrix-a case-control study in British Columbia, Canada. *Scand J Work Environ Health* 33(6): 454-464.

[5] Böhm F, Schmid D, Denzinger S, Wieland WF, Richter E. 2011. DNA adducts of *ortho*-toluidine in human bladder. *Biomarkers* 16(2): 120-128.

findings do not provide conclusive evidence of covalent binding of *ortho*-toluidine to DNA.

- One Panel member suggested adding in the monograph an analysis of combined incidence of mammary fibroadenoma and adenocarcinoma observed in F344 male rat exposed to *ortho*-toluidine (Hecht *et al.*1982). The member cited a reference,[6] which notes that mammary adenocarcinoma may also arise from foci of atypia in adenoma or fibroadenoma.

NTP Response: An analysis of the combined mammary tumors (fibroadenoma and adenocarcinoma) is now included in Table 4-4, and the text is revised to note that occasionally mammary adenocarcinoma arise within a fibroadenoma.

Comments and NTP's response related to improving the clarity of the RoC monograph.

Panel Comments

- Use consistent units for reporting *ortho*-toluidine hemoglobin adducts (Hb) in the exposure section (primarily Table 1-4, *ortho*-Toluidine in urine and Hb adducts in different populations): normal Hb values can be used to convert between ng/liter blood and pg/g Hb.
- Clarify in the mechanistic section that the increased incidences of the rare urinary bladder tumors observed in male rats were not statistically significant.
- Correct the description of the Watanabe *et al.* 2010[7] study in the mechanistic section, overall cancer evaluation, and the substance profile; copper is not required for induction of 8-oxodG adducts in human leukemia cells by *ortho*-nitrosotoluene. The text states that oxidative damage occurred only in the presence of Cu (II) and NADH.
- The draft monograph states that subcutaneous injection in experimental animal studies is a less relevant route of exposure for human carcinogenicity; however, given the importance of dermal exposure in humans, it may be a relevant route for *ortho*-toluidine exposure.

NTP Response: The NTP concurs with the Panel recommendations and has revised the monograph accordingly.

Comments and NTP response related to enhancing the completeness of the monograph

Panel Comments

- Add additional exposure information in the properties and exposure section of the cancer evaluation document and substance profile, including information on dermal exposure and absorption, the importance of exposure from second-hand

[6] Boorman GA, Wilson JT, van Zwieten MJ, Eustis SL. 1990. Chapter 19, Mammary Gland, In: Boorman GA, Eustis SL, Elwell, MR, Montgomery CA, Jr., MacKenzie WF (eds). Pathology of the Fischer Rat, pp. 291-313.

[7] Watanabe C, Egami T, Midorikawa K, Hiraku Y, Oikawa S, Kawanishi S, Murata M. 2010. DNA damage and estrogenic activity induced by the environmental pollutant 2-nitrotoluene and its metabolite. *Environ Health Prev Med* 15(5): 319-326.

smoke, potential exposure from tattoos, trends of prilocaine use in dental care, and other compounds that metabolize *ortho*-toluidine. One Panel member suggested that a structured search, such as Chemical Abstracts Service SciFinder, could be used to identify products that could metabolically release *ortho*-toluidine.

NTP Response: The NTP concurs with these recommendations and has added most of this information to the revised draft monograph. No new information was found on trends of prilocaine use in the Untied States. A structured search for other compounds releasing *ortho*-toluidine was not done because it goes beyond the scope of the exposure section of the monograph. The RoC monograph is not meant to be a comprehensive review of all the exposure information on a specific substance and instead focuses on information needed to determine whether a significant number of people residing in the Untied States are exposed to *ortho*-toluidine, the major sources of exposure, and exposure information needed to understand the human cancer section.

- Add the following mechanistic related information to the mechanistic section and overall cancer evaluation of the cancer evaluation component, and/or the substance profile: (1) findings from two additional genotoxicity studies (Sekihashi *et al.* 2002[8] and Brennan and Schiestl 1999[9]), (2) cellular transformation to the summary of the list of genetic effects induced by *ortho*-toluidine, (3) a table (structural activity relationship, SAR) comparing the tumor sites by species and sex of *ortho*-toluidine and two structurally related compounds (*ortho*-nitrotoluene, and *ortho*-nitrosotoluene), and (4) a summary of the adsorption, distribution, metabolism, and excretion findings in humans and animals, i.e., urinary metabolites *N*-acetyl-*o*-toluidine, tissue distribution in rodents, and the sites of metabolism including alkyl oxidation, to the substance profile.

NTP Response: The NTP concurs with the Panel recommendations and has added most of this information to the revised draft monograph. The NTP did not include a SAR table in the monograph because the key findings from *ortho*-nitrosotoluene, as it relates to informing the mechanisms of urinary carcinogenicity of *ortho*-toluidine, are already discussed in the document. The NTP has expanded the discussion of the metabolism data in the draft substance profile to include information suggested by the Panel, which is discussed in greater detail in the cancer evaluation component.

[8] Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, Knasmüller S, Tsuda S, Sasaki YF. 2002. Comparative investigation of multiple organs of mice and rats in the comet assay. *Mutat Res.* 517 (1-2): 53-75.

[9] Brennan RJ, Schiestl RH. 1999. The aromatic amine carcinogens o-toluidine and o-anisidine induce free radicals and intrachromosomal recombination in *Saccharomyces cerevisiae*. *Mutat Res* 430(1): 37-45.

Pentachlorophenol and By-Products of its Synthesis

The Draft RoC Monograph for Pentachlorophenol and By-products of its Synthesis was peer reviewed at a public meeting held December 12–13, 2013, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. The NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions, and (2) scientific and technical peer-review comments related to scientific issues and to improve the technical accuracy, clarity, and objectivity of the monograph. The Panel also provided several editorial comments, which are not included in the NTP response to the Peer-Review Report. These comments were also carefully considered in preparing the revised draft monograph.

The Panel's Recommendations and NTP Response

Panel Recommendations

The NTP's conclusion regarding U.S. exposure

The Panel agreed that a significant number of people in the United States are exposed to pentachlorophenol and by-products of its synthesis.

The NTP's preliminary listing decision for pentachlorophenol and by-products of its synthesis in the RoC

The Panel recommended unanimously (10 yes, 0 no, 0 abstentions) to change NTP's preliminary policy decision in the draft monograph from *known to be a human carcinogen* to *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity of pentachlorophenol, and of pentachlorophenol and by-products of its synthesis, from studies in experimental animals, and supporting mechanistic evidence.

NTP response: The NTP concurs with the Panel that pentachlorophenol and by-products of its synthesis is *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity from studies in experimental animals, and supporting mechanistic evidence. However, the RoC does not concur with the Panel's conclusion of sufficient evidence of carcinogenicity of pentachlorophenol alone from studies in experimental animals.

The NTP's conclusion regarding the level of evidence for carcinogenicity from human cancer studies

The Panel recommended unanimously (10 yes, 0 no, 0 abstentions) changing the NTP's level of evidence conclusion in the draft monograph from *sufficient* to *limited* evidence of carcinogenicity in human cancer studies of pentachlorophenol and by-products of its synthesis. Limited evidence indicates a causal association between pentachlorophenol and by-products of its synthesis and non-Hodgkin lymphoma (NHL) is credible, but alternative explanations such as chance, bias, or confounding factors could not be adequately excluded.

NTP Response: NTP concurs with the Panel that the evidence for an association between NHL and exposure to pentachlorophenol and by-products of its synthesis is primarily based on one large, high-quality study of Canadian sawmill workers (Demers *et al.* 2006[10]) with support from a cohort study of Michigan pentachlorophenol producers (Collins *et al.* 2009[11]). The quality of the evidence for carcinogenicity from the other studies is lower. Some Panel members noted that the evidence from the Michigan pentachlorophenol producers study points to dioxin-like activity and did not think the study supports the carcinogenicity of pentachlorophenol alone. The NTP acknowledges this is a possibility and believes the epidemiologic studies cannot separate effects of pentachlorophenol alone from those of its by-products; thus, the NTP believes the study supports the definition of the candidate substance. Due to the limited number of high-quality studies, chance and confounding across studies cannot be adequately excluded. The draft monograph was revised to clarify the limitations of the quality of evidence.

The NTP's conclusion regarding the level of evidence for carcinogenicity from studies in experimental animals

The Panel recommended (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. The Panel also unanimously recommended (10 yes, 0 no, 0 abstentions) that there is sufficient evidence of carcinogenicity of pentachlorophenol alone based on studies in experimental animals.

NTP Response: The NTP agrees with the conclusion of sufficient evidence of carcinogenicity of pentachlorophenol and by-products of its synthesis from studies in experimental animals. This conclusion is based on the collective evidence from studies in mice using two different preparations of pentachlorophenol with different concentrations of dioxin by-products: technical grade pentachlorophenol (90% pure) and Dowicide EC-7 (91% pure) and a study in rats using "pure" (analytical grade) pentachlorophenol (99%).[12]

The NTP does not have enough confidence in the body of evidence from studies in experimental animals to support the Panel's conclusion of sufficient evidence of carcinogenicity of pentachlorophenol alone (excluding by-products of its synthesis). The NTP concurs with the Panel that the collective evidence from the cancer studies (stop-

[10] Demers PA, Davies HW, Friesen MC, Hertzman C, Ostry A, Hershler R, Teschke K. 2006. Cancer and occupational exposure to pentachlorophenol and tetrachlorophenol (Canada). *Cancer Causes Control* 17(6): 749-758.

[11] Collins JJ, Bodner K, Aylward LL, Wilken M, Swaen G, Budinsky R, Rowlands C, Bodnar CM. 2009. Mortality rates among workers exposed to dioxins in the manufacture of pentachlorophenol. *J Occup Environ Med* 51(10): 1212-1219.

[12] NTP. 1989. Toxicology and Carcinogenesis Studies of Two Pentachlorophenol Technical-Grade Mixtures (CAS No. 87-86-5) Administered in B6C3F1 Mice (Feed Studies). *Technical Report Series No. 349. NIH Publication No. 89-2804.* Research Triangle Park, NC: National Toxicology Program. 267 pp. NTP. 1999. Toxicology and Carcinogenesis Studies of Pentachlorophenol (CAS No. 87-86-5) in F344/N Rats (Feed Studies). *Technical Report Series No. 483. NIH Publication No. 99-3973. Research Triangle Park, NC: National Toxicology Program. 184 pp.*

exposure and 2-year bioassay) in rats using pure pentachlorophenol (excluding by-products of its synthesis) is not strong enough by itself to support a call of sufficient evidence of carcinogenicity. The Panel considered the cancer studies in mice using Dowicide EC-7, which has lower amounts of dioxin-like by-products than technical grade pentachlorophenol, supportive of the carcinogenicity of pure pentachlorophenol. The NTP notes that although dioxin-like by-products are present at lower concentrations, Dowicide EC-7 is not free of dioxin-like by-products. In addition, tetrachlorophenol, a by-product present in Dowicide EC-7 at a higher concentration (approximately 9%) than in technical grade (approximately 3.8%), has not been tested for carcinogenicity. Evidence from chlorophenol metabolism studies support the potential formation of a reactive metabolite tetrachlorohydroquinone from tetrachlorophenol, which may lead to tumor formation. Finally, cancer studies using pure pentachlorophenol were conducted in rats and not mice, and Dowicide EC-7 studies were conducted in mice and not rats, which limit direct comparisons of the two preparations.

Scientific and technical peer-review comments on the draft RoC monograph

The Panel provided comments regarding the Draft RoC Monograph for Pentachlorophenol and By-products of its Synthesis on scientific issues and that would add to its clarity and completeness. The specific comments and NTP's response to the comments are discussed below.

Comments and NTP's response related to scientific issues in the RoC monograph

- The Panel noted some inconsistency in the initial inclusion or exclusion of studies presenting data on chlorophenols as a group only. The draft monograph included the Australian[13] case-control study on malignant lymphoma and soft tissue sarcoma and the series of New Zealand[14] case-control studies of multiple myeloma, NHL, and soft tissue sarcoma but not other epidemiologic studies of exposure to mixed chlorophenols.

  NTP Response: The NTP originally included the Australian and New Zealand studies in the monograph because they provide limited information on exposure to pentachlorophenol (e.g., the authors noted that pentachlorophenol was the predominant chlorophenol used in their country), whereas the other studies on mixed chlorophenols had little to no exposure-specific information. The NTP concurs with the Panel's comment that the inclusion of the Australian and New Zealand case-control studies creates some ambiguity. The studies have now been excluded from the assessment at the initial inclusions/exclusion step.

[13] Smith JG, Christophers AJ. 1992. Phenoxy herbicides and chlorophenols: a case control study on soft tissue sarcoma and malignant lymphoma. *Br J Cancer* 65(3): 442-448.

[14] Smith AH, Pearce NE, Fisher DO, Giles HJ, Teague CA, Howard JK. 1984. Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. *J Natl Cancer Inst* 73(5): 1111-1117. Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA. 1986. Case-control study of multiple myeloma and farming. *Br J Cancer* 54(3): 493-500. Pearce NE, Sheppard RA, Smith AH, Teague CA. 1987. Non-Hodgkin's lymphoma and farming: an expanded case-control study. *Int J Cancer* 39(2): 155-161.

- One Panel member recommended that the series of Swedish case-control studies[15] on soft tissue sarcoma and lymphoma be excluded from the assessment because of limitations in the exposure assessment and the lack of scientific plausibility for the findings of a high risk estimate for NHL in the 1994 study.

NTP Response: The NTP concurs that the exposure assessment of the Swedish case-control studies is limited. The assessment in the draft monograph gave less weight to this study. In general, the NTP approach for including studies in an RoC evaluation leans towards being inclusive, noting study limitations and whether the limitations would generally lead to an under or over estimate of the risk estimate, or to that the direction of the bias is not determinable. Thus, the study remains in the revised draft monograph, with the methodological limitations clearly noted.

- Some Panel members thought that the NIOSH[16] study of pentachlorophenol producers distracts from the evidence supporting an association between pentachlorophenol and NHL. The NIOSH study includes the pentachlorophenol workers at the Michigan plant reported by Collins *et al.* (2009)[17]. In the NIOSH study, the risk estimate among workers exposed to pentachlorophenol was lower than that for workers exposed to both pentachlorophenol and trichlorophenol (TCP), whereas in the Michigan study, the risk estimate was higher among pentachlorophenol workers than workers exposed to both chlorophenols. In addition, the NIOSH study did not find a relationship between employment duration in pentachlorophenol or TCP departments.

NTP Response: The NTP does not believe that the NIOSH study provides evidence against an association between pentachlorophenol and NHL. The NTP has added text in the revised monograph to support its position, including more information on the overlap between the NIOSH and Michigan studies. The NIOSH study classified a larger number of the production workers at the Michigan plant as being exposed to both pentachlorophenol and TCP than the Michigan study. This is probably because the exposure assessments for TCP differed in the two studies: both studies classified the workers as also being exposed to TCP if they worked directly with TCP; however, the NIOSH study also considered workers as exposed to TCP if they worked in a building where TCP processes were co-located. Most (77%) of the pentachlorophenol and TCP exposed workers in the entire NIOSH cohort (at all plants) were from the

[15] Hardell L, Eriksson M, Degerman A. 1994. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. *Cancer Res* 54(9): 2386-2389. Hardell L, Eriksson M, Degerman A. 1995. Meta-analysis of four Swedish case-control studies on exposure to pesticides as risk-factor for soft-tissue sarcoma including the relation to tumor-localization and histopathological type. *Int J Oncol* 6(4): 847-851. Hardell L, Eriksson M, Nordström M. 2002. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma* 43(5): 1043-1049.

[16] Ruder AM, Waters MA, Butler MA, Carreón T, Calvert GM, Davis-King KE, Schulte PA, Sanderson WT, Ward EM, Connally LB, Heineman EF, Mandel JS, Morton RF, Reding DJ, Rosenman KD, Talaska G. 2004. Gliomas and farm pesticide exposure in men: the upper midwest health study. *Arch Environ Health* 59(12): 650-657.

[17] Collins JJ, Bodner K, Aylward LL, Wilken M, Swaen G, Budinsky R, Rowlands C, Bodnar CM. 2009. Mortality rates among workers exposed to dioxins in the manufacture of pentachlorophenol. *J Occup Environ Med* 51(10): 1212-1219.

Michigan plant. Thus, the biomonitoring data on pentachlorophenol-exposed and pentachlorophenol and TCP-exposed workers reported by Collins *et al.* (2008)[18] for the Michigan study are relevant to the entire NIOSH cohort. This study found that pentachlorophenol-exposed workers had only a small increase in serum levels of the TCP by-product 2,3,7,8-TCDD (dioxin) as compared to unexposed workers, whereas a much larger increase was observed for workers exposed to both pentachlorophenol and TCP. Furthermore, no association between TCP and NHL was found among TCP workers in the Michigan plant, which argues against potential confounding by the TCP by-product 2,3,7,8-TCDD[19]. The NTP also believes the employment-duration analysis in the NIOSH study is uninformative, rather than negative evidence, because of the uncertainty of the utility of employment duration as a metric for pentachlorophenol exposure, the small number of workers, and the potential of bias from the healthy worker survival effect.

The following comments are related to improving the clarity and technical accuracy of information reported in the draft monograph.

- In the discussion of immunosuppression in studies in mice (Section 5.2.2) remove any speculation from the discussion of immune effects.

NTP Response: Conclusions based on the data reported were added to the discussion of immune effects; comments based on speculation were removed.

- Modify Figure 5-1, "scheme of pentachlorophenol adduct formation: reactivity of phenoxyl radical toward dG", to include in the figure that only one adduct (O bonded C8-dG adduct) was found with either deoxyguanosine (dG) or calf thymus-DNA (CT-DNA) reacting with horseradish peroxidase (HRP) and cite Dai *et al.* (2003, 2005)[20] Delete Figure 5-2, "postulated mechanism for 4"-hydroxy-1,N^2-benzetheno-dG formation" as it is incorrect.

NTP Response: Figure 5-1 has been modified as suggested and Figure 5-2 deleted from the monograph.

The following Panel comments are related to enhancing the completeness of the monograph.

- Add additional exposure information on imports, if available, including a table of the production by-products of pentachlorophenol identified by company.

NTP Response: Information on imports is found in Table 1-3. Additional exposure information on imports, if available, has been added and a table of pentachlorophenol

[18] Collins JJ, Bodner K, Haidar S, Wilken M, Burns CJ, Lamparski LL, Budinsky RA, Martin GD, Carson ML. 2008. Chlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyl profiles of workers with trichlorophenol and pentachlorophenol exposures. *Chemosphere 7*3(1 Suppl): S284-289.
[19] Collins JJ, Bodner K, Aylward LL, Wilken M, Bodnar CM. 2009. Mortality rates among trichlorophenol workers with exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Am J Epidemiol* 170(4): 501-506.
[20] Dai J, Wright MW, Manderville RA. 2003. An oxygen-bonded C8-deoxyguanosine nucleoside adduct of pentachlorophenol by peroxidase activation: evidence for ambient C8 reactivity by phenoxyl radicals. *Chem Res Toxicol* 16: 817-821. Dai J, Sloat AL, Wright MW, Manderville RA. 2005. Role of phenoxyl radicals in DNA adduction by chlorophenol xenobiotics following peroxidase activation. *Chem Res Toxicol* 18: 771-779.

production by-products of synthesis by U.S. companies has been added to the draft revised monograph.

- Add information (if available) on production and emplacement of pentachlorophenol-treated telephone poles and railroad ties in the environment.
- Add information (from NHANES data) on children having higher levels of pentachlorophenol than adults and on adults noting pentachlorophenol was detectable in 71.6% of the general population and include this information in the discussion.
- Add more information about half-life of pentachlorophenol in humans (if available) and on what factors could account for the variations in half-life and clearance rates.

NTP Response: This information has been added to the revised draft monograph. Available data on half-life in humans is in Table 2-7. Additional information on factors that may affect half-life and clearance rates has also been added.

- Include in the monograph the citation, Mehmood *et al.* (1996)[21], demonstrating conversion by human P450 liver enzyme, CYP3A4 of pentachlorophenol to tetrachlorohydroquinone, a reactive metabolite,.
- Summarize information on mechanism of liver carcinogenesis from other relevant data (section 5), including data on metabolism of pentachlorophenol to reactive metabolites, induction of reactive oxygen species, formation of O bonded C8-dG adducts, and glutathione depletion, and include in the overall cancer evaluation (Section 6). Add an additional reference (Tasaki *et al.* 2012)[22] on the involvement of *Nrf2* in pentachlorophenol-induced tumor progression.

NTP Response: More detail on the metabolism of pentachlorophenol and mechanism of liver cancer and discussion of *Nrf2* involvement was added to the monograph.

- In the draft Substance Profile add discussion of the current NHANES data, including that exposure was higher in children than in adults, and include information on the levels of contaminants in technical grade pentachlorophenol and Dowicide EC-7, perhaps noting worst-case dioxin toxic equivalents. Also correct information on DNA adducts and immunosuppression and include work of Mehmood *et al.* (1996) in the discussion of pentachlorophenol metabolism by human liver enzymes.

NTP Response: A discussion of the current NHANES data was added; Mehmood et al. (1996) was included and information on DNA adducts and immunosuppression corrected in the Substance Profile. Concentrations of pentachlorophenol in technical grade and Dowicide EC-7 and text stating that the technical grade contains levels of dioxin-like activity than Dowicide EC-7 were provided in the draft substance profile.

[21] Mehmood Z, Williarnson MP, Kelly DE and Kelly SL. 1996. Metabolism of organochlorine pesticides: the role of human cytochrome P450 3A. *Chemosphere* 33: 759-769.

[22] Takaski M, Kurolwa Y, Inoue T, Hibi D, Matsushita K, Kijima A, Maruyama S, Nishikawa A, Umemura T. 2014. Lack of *nrf2* results in progression of proliferative lesions to neoplasms induced by long-term exposure to non-genotoxic hepatocarcinogens involving oxidative stress. *Exp Tox Path* 66: 19-26.

Detailed listings of by-products, including the dioxin-like equivalents, and other chemicals for both preparations are in Appendix F of the cancer evaluation component of the monograph. The substance profile of the revised monograph will reference the cancer evaluation component for this information.