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Re: Draft Report on Carcinogens Monographs for Pentachlorophenol and By-Products of Its Synthesis; Availability of Documents; Request for Comments; Notice of Meeting, 78 Fed. Reg. 51,733 (Aug. 21, 2013)

Submitted on Behalf of the Pentachlorophenol Task Force

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Introduction

The National Toxicology Program (NTP 2013) Peer-Review Draft: Report on Carcinogens Monograph on Pentachlorophenol and By-Products of Its Synthesis (hereafter NTP Listing Document) concludes that pentachlorophenol (PCP) and by-products of its synthesis should be considered as "known" to cause cancer in humans (i.e., specifically non-Hodgkin's lymphoma; NHL). With respect to inclusion of PCP synthesis by-products it is stated, "*Evidence that exposure to pentachlorophenol includes exposure to by-products of its synthesis comes from biomonitoring studies. The pentachlorophenol by-products most commonly found in serum samples are 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin, and octachlorodibenzo-p-dioxin, but not 2,3,7,8-TCDD, which is not a by-product of the pentachlorophenol synthetic process used in the United States*." This statement implies that the PCP synthesis by-products contribute to the overall carcinogenicity of PCP. However, if PCP itself, without consideration of synthesis by-products, is not a human carcinogen, and there is no evidence that PCP byproducts are themselves human carcinogens, then it is difficult to understand the rationale for concluding that PCP and the byproducts of its synthesis are known to cause cancer in humans. Furthermore, while 2,3,7,8-TCDD (hereinafter "TCDD") is not a by-product of PCP synthesis in the United States (U.S.), it clearly confounds a number of epidemiological studies due to its presence in the substances to which certain cohorts were exposed. However, as documented in the NTP Listing Document, TCDD exposure cannot be attributable to commercial PCP used in the U.S. In addition, the confusion surrounding whether TCDD is a PCP synthesis by-product, PCP contaminant, or confounding factor in the PCP

epidemiological data set has substantial implications with respect to how this body of evidence was assessed and weighed as support for the overall conclusion of the NTP Listing Document for PCP and synthesis by-products.

Importantly, since TCDD is not a by-product of PCP synthesis in the U.S. its inclusion in the NTP Listing Document introduces a number of interpretative and logical issues. Foremost is why TCDD, listed by IARC as a "known" cause of all cancers combined with emphasis on NHL (Baan et al. 2009), is at all relevant to an assessment of the carcinogenicity of Pentachlorophenol and By-Products of Its Synthesis. Moreover, absent robust data demonstrating that the actual byproducts of PCP synthesis are themselves known human carcinogens, it is unclear how including them in the listing strengthens a conclusion as to overall carcinogenicity of PCP where PCP itself is not a known human carcinogen. This has recently and clearly been established by the EPA/IRIS (2009) Toxicological Review of Pentachlorophenol which examined essentially the same data set that has been reviewed in the NTP Listing Document concluding only that PCP was *likely to be carcinogenic to humans.*

Even if the intent is to list PCP contaminated by TCDD from sources manufactured and potentially still used in a limited number of Asian countries, the toxicity data available on TCDD has been robust for many years and it is unknown why it is necessary to consider the known association of TCDD with NHL in the NTP Listing Document. A critical assessment of the data on PCP alone, including non-TCDD contaminants, using the concepts embodied in the EPA (2005) Guidelines for Cancer Risk Assessment and now embraced in the NTP Office of Health Assessment and Translation (OHAT) Draft OHAT Approach For Systematic Review And Evidence Integration For Literature-Based Health Assessments (2013) should be the preferred methodology upon which a judgment on the potential human carcinogenicity of PCP should be based. Noteworthy, with respect to this issue, in the NTP (2013) Protocol: Evaluation of Human Cancer Studies on Exposure to Pentachlorophenol and By-products of its Synthesis for the Report on Carcinogens, while all but one of the existing guidelines based on the Bradford Hill "causation criteria" were explicitly embraced, i.e., "*strength of the association, consistency across studies, evidence of an exposure-response gradient, and temporality of exposure*" there was one glaring exception. Recognizing that this NTP (2013) Protocol was directed at an evaluation of the epidemiological data, inexplicitly missing was the one causation criteria that goes to the issue of common sense, i.e., biological plausibility. If this criterion is not important, particularly with respect to informing the epidemiology data, why is there extensive discussion about the animal carcinogenicity data on PCP and contaminants, mutagenicity data and potential mode of action data in the NTP Listing Document?

The overall conclusion in the NTP Listing Document is presumably based on (1) animal carcinogenicity data for PCP and synthesis by-products (2) *in vitro* and *in vivo* mutagenicity data, and (3) human epidemiology studies. These comments addresses the above issues in the context of (1) whether PCP alone (or including non-TCDD contaminants) is capable of causing cancer in animals or humans and (2) if not, whether it is rational or appropriate to conclude that synthesis by-products other than TCDD

might play a role in animal or human PCP carcinogenicity. Clearly, to the extent that any epidemiological study on PCP-exposed cohorts includes simultaneous exposure to TCDD, such data should not be relied upon for the listing of PCP as a potential or known human carcinogen for the same endpoint "known" to be caused by TCDD.

In reality, it appears that the assumed relevance of the TCDD dataset to the carcinogenicity of PCP and the actual byproducts of its synthesis confounds not only the interpretation of the epidemiological data, but now also the process of assessing the potential carcinogenicity of PCP alone. In order to maintain the integrity of the NTP listing process and because there are sufficient data to support this, the listing of PCP as a potential human carcinogen should be based only on PCP and not include synthesis by-products that are not present in the commercially available PCP used in the U.S.

The NTP Listing Document's Treatment of 2,3,7,8-TCDD

The NTP Listing Document states (pp. iii-iv) that, "*One of the key issues identified in the concept document concerns differentiating effects of pentachlorophenol from its contaminants in both the cancer studies in humans and experimental animals. In order to receive public and scientific input on this matter, the ORoC held a webinar titled, 'Human cancer studies on exposure to pentachlorophenol (PCP): Differentiating potential cancer effects of PCP exposure from effects due to occupational co-exposures or PCP contaminants' on April 11, 2013. The ORoC also convened an information group consisting of several scientists within and outside of NTP with substance-specific expertise to independently review the experimental animal data.* ***Based on this input, the NTP has defined the candidate substance as 'pentachlorophenol and by-products of its synthesis***.'" (emphasis added).

It is essential to distinguish between the terms "contaminant" and "by-products of synthesis" and "co-exposures," all of which are used, seemingly interchangeably, in the NTP Listing Document, and to indicate which term refers to agents considered as potential confounders. Of particular importance is the Draft's treatment of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This compound has been classified by The International Agency for Research on Cancer (Baan et al., 2009) as a "known human carcinogen" based on sufficient evidence in humans for all forms of cancer combined and on more limited evidence in humans of causing non-Hodgkin lymphoma (NHL), soft tissue sarcoma (STS) and lung cancer, three forms of cancer reported to be associated with PCP.

2,3,7,8-TCDD is one by-product of the synthesis of pentachlorophenol (PCP) using alkaline hydrolysis of hexachlorobenzene (HCB), a process not used in the US but sometimes used in a few Asian countries and more widely used in Europe prior to the 1990s. The Listing Document states that although the alkaline hydrolysis of HCB results in the formation of 2,3,7,8-TCDD, "… *2,3,7,8-TCDD has rarely been detected in commercial preparations of pentachlorophenol…thus* ***the presence of this molecule in***

a pentachlorophenol preparation is considered to be a contaminant *rather than a by-product of its synthesis*."(p. 4) (emphasis added). This statement implies that 2,3,7,8-TCDD should be considered a possible confounder, and elsewhere in the draft, TCDD appears to be treated as a potential confounder (e.g., Table 3-2a. Occupational co-exposure and methods relevant for evaluating confounding, pp. 47-48). And, in some of the key epidemiologic studies, 2,3,7,8-TCDD exposure may have occurred, not because TCDD was a by-product of PCP synthesis but because TCDD contamination from other manufacturing processes was present.

The Listing Document later seems to indicate that the definition of "*pentachlorophenol and the by-products of its synthesis*" includes 2,3,7,8-TCDD as a by-product and states that "*2,3,7,8-TCDD* ***is not considered to be a contaminant of pentachlorophenol production***" (p. 75, emphasis added). Thus, further clarification of the treatment of 2,3,7,8-TCDD in the Listing Document is needed. Because 2,3,7,8-TCDD is already listed as an established human carcinogen, a consideration of "PCP and the by-products of its synthesis" may be reasonable only if this term means "PCP and the by-products of its synthesis, excluding 2,3,7,8-TCDD."

Review of Animal Data

While there are numerous cancer bioassays on PCP, the most relevant is the study by Chhabra et al. (1999) conducted by NIEHS/Battelle. This study was undertaken even though a previous two-year study in SD rats (Schwetz et al. 1978) with 96.4% PCP while not showing any evidence of carcinogenicity had been criticized for some design deficiencies, [i.e., small group sizes (25 animals/dose) and dose levels (1, 3, 10 & 30 mg/kg)]. However, the highest dose level was associated with mild signs of toxicity including depressed body weight gain and increased serum glutamic pyruvic transaminase activity in both sexes. This suggests that sufficient toxicity occurred to induce one or more of the mode of action events as discussed below.

In the Chhabra et al. study 50 male and 50 female F344 rats were administered >99% pure PCP in the diet at 200, 400 or 600 mg/kg for two years. A stop-exposure study was also included in which 60 male and 60 female animals received 1000 mg/kg in the diet for one year followed by the control diet for the remainder of the 2 year study. There was no evidence of carcinogenicity in male or female rats at dietary levels of 200, 400 or 600 mg/kg for 2 years. After 2 years in the stop-exposure animals there was evidence of malignant mesothelioma in the tunica vaginalis in nine 1000 ppm males and one control male. However, as described by Maronpot et al. (2009) tunica vaginalis mesothelioma responses are very specific to male F344 rats which bring into question their relevance for extrapolation to other species, especially humans. This is particularly the case since similar tumors are not observed in females or mice in conventional cancer bioassays and have not been reported in other rat strains

Nasal squamous cell carcinoma was observed in five 1000 mg/kg males and one control male. Given the well-established propensity of rats to sniff their food and the unlikely production of this tumor type from systemic delivery to the nasal epithelium,

this finding was likely due to direct inhalation and high-dose cytotoxicity of the 1000 mg/kg PCP dose. There was no evidence of PCP carcinogenic activity in stop-exposure females. It was concluded, therefore, that there was some evidence of PCP carcinogenic activity in male animals. The likely relevance of these tumors are further discussed below in the section on potential modes of action of PCP-induced carcinogenesis. However, it should be noted that the stop-study dose of 1000 mg/kg is almost twice that of the highest dose (i.e., 600 mg/kg) that had no effect on tumor development, including nasal tumors or tunica vaginalis mesothelioma. As discussed below in the mode-of-action section, this suggests that the 1000 mg/kg dose was so high that it caused substantial cytotoxicity-driven effects not produced by the 600 mg/kg dose in the full 2-year study. Finally, the use of a stop-study dose higher than the dose administered for 2 years contradicts the protocol used in all of the recent NTP chronic carcinogenicity studies conducted on various dioxins, furans and PCBs. In all of these studies the stop-study dose was the same as the highest dose used in the full 2-year study. This is due to the fact that using a different stop-study dose precludes an informed comparison with the full study results as is evident with the flawed design of the Chhabra et al. (1999) study.

The other studies reviewed in the NTP Listing Document with PCP formulations of approximately 90% purity should not be afforded weight in an evaluation of PCP given the fact the PCP alone (i.e., >99% pure) did not induce tumors in the Chhabra et al. (1999) study. The results of this study can only be interpreted as demonstrating that PCP alone is not capable of causing tumors in rats. While the animal cancer bioassay data conducted with ≈ 90% pure PCP demonstrate that this chemical mixture is a multisite, multispecies carcinogen with a high degree of confidence the same cannot be said about >99% pure PCP. The available data suggest that >99% pure PCP is not carcinogenic in animal studies.

While the document concludes that "*There is sufficient evidence for the carcinogenicity of pentachlorophenol and by-products of its synthesis in experimental animals*" there is essentially no evidence that PCP alone is carcinogenic. The fact that the listing of PCP and contaminants is based on exposure-related malignant and/or a combination of malignant and benign neoplasms of various organs is not relevant with respect to PCP alone.

As noted above, the PCP by-products most commonly found in serum samples are 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, and octachlorodibenzo-*p*-dioxin (but not 2,3,7,8-TCDD). If this is meant to infer that these by-products are the chemicals likely responsible for the carcinogenic effects in either animals or humans following exposure to technical grade PCP, there is no relevant evidence to support this conclusion.

The only one of the above chemicals studied for potential carcinogenicity in an animal bioassay is hexachlorodibenzo-p-dioxin. In a highly relevant 2-year study a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins (HCDD) was assessed by NCI (1980) for possible carcinogenicity by dermal application of a suspension of this

hexachlorodioxin mixture to Swiss-Webster mice. As concluded by NCI (1980) "*Under the conditions of this bioassay, HCDD was not carcinogenic for male or female Swiss-Webster mice*." It is noteworthy that this study was not cited or relied upon in the NTP Listing Document while the essentially irrelevant gavage study on the same mixture was.

These results of the dermal study on hexachlorodibenzo-p-dioxin have substantial implications for the potential carcinogenicity for the other PCP synthesis by-products 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, and octachlorodibenzo-*p*-dioxin. While neither has been tested for carcinogenicity, with Toxic Equivalence Factors (TEFs) of 0.1, 0.01 and 0.0003 for the hexa, hepta, and octadioxins, respectively, there is no reason to suspect that the heptachloro or octachloro compounds would have any carcinogenic activity. TEF's represent the potency of higher chlorinated dioxins relative to the potency of TCDD. Since these are the most commonly found dioxin contaminants in worker serum samples, it can only be concluded that none would contribute to potential carcinogenicity as by-products of PCP synthesis. This issue is not mentioned or discussed in the NTP Listing Document.

A companion bioassay on a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins (HCDD) was tested for possible carcinogenicity by administering the test material by gavage to Osborne-Mendel rats and B6C3F1 mice for 104 weeks (NCI 1980. In this study, Fifty rats and 50 mice of each sex were administered HCDD by gavage 2 days per week for 104 weeks at doses of 1.25, 2.5, or 5 ug/kg/wk for rats and male mice and 2.5, 5, or 10 ug/kg/wk for female mice. Seventy-five rats and 75 mice of each sex served as vehicle controls. As concluded by NCI (1980) "*Under the conditions of this bioassay, HCDD administered by gavage was carcinogenic, causing increased the incidences of hepatocellular carcinomas or neoplastic nodules in female Osborne-Mendel rats and inducing hepatocellular carcinomas and adenomas in male and female B6C3F1 mice. HCDD was not demonstrated to be carcinogenic for male rats*."

However, as noted in the NTP (2013) Listing Document, "*Human exposure to pentachlorophenol occurs in occupational settings through **dermal contact** with the substance or with treated wood products and **via inhalation** of affected workplace air*." [emphasis added] While it is rare to have cancer bioassay data on the same compound by different routes of exposure, it is clear that only one of the NCI studies is relevant to the present issue. Consequently, since human exposure occurs exclusively through either dermal (i.e., *The major route of exposure for workers using pentachlorophenol to treat wood is dermal* or inhalation exposure, the results of the NCI gavage study are of less relevance for assessing the potential human risks than the NCI dermal exposure study since this is not a potential route of exposure of humans to PCP and therefore to hexachlorodibenzo dioxins. The inability of dermal exposure to hexachlorodibenzodioxin to cause tumors in in the NCI (1980) study suggests that this more relevant route of exposure should be the one to use when inferring potential human carcinogenicity to the hexachloro dioxin PCP synthesis by-products from the animal data.

Brief Review of *In Vitro* and *In Vivo* Mutagenicity Data

While there are abundant data on the potential *in vitro* mutagenic effects of PCP, such data are of limited relevance with respect to inferring potential *in vivo* effects. However, as summarized in Table E-6 of the NTP (2013) Listing Document (i.e., *In vivo* studies of cytogenetic effects of pentachlorophenol in rodents) the results are overwhelmingly negative particularly for 99% pure PCP. This is confirmed by Table 4-19 from the EPA/IRIS (2010) assessment of PCP which demonstrates mostly negative results in *in vivo* genotoxicity studies of PCP. Consequently, it is implausible that early mutation plays a role in PCP carcinogenicity. This is particularly the case with >99% pure PCP, which, as discussed above, is not carcinogenic following two-year exposure at the highest dose of 600 mg/kg.

Table 4-19 Summary of selected *in vivo* genotoxicity studies of PCP

Endpoint	Result	Reference
Micronucleus formation in mice	Negative	NTP (1999); Xu (1996)
Micronucleus formation in rats	Negative	NTP (1999)
Sex-linked recessive lethal mutation in *Drosophila melanogaster*	Negative	Vogel and Chandler (1974)
Point mutations in p53 gene in hepatocytes	Positive	Yin et al. (2006)
Tumor multiplicity in Ha-ras transgenic mice	Positive	Spalding et al. (2000)
CAs in human lymphocytes	Weakly positive	Bauchinger et al. (1982)
CAs in human lymphocytes	Negative	Ziemsen et al. (1987)
CAs in male rat hepatocytes	Negative	Daimon et al. (1997)
SCE in human lymphocytes	Negative	Bauchinger et al. (1982)
SCE in human lymphocytes	Negative	Ziemsen et al. (1987)
SCE in male rat hepatocytes	Weakly positive	Daimon et al. (1997)

Brief Review of Potential Mode of Action Data

Both the NTP Listing Document as well as the EPA/IRIS (2010) review of PCP addressed the issue of potential mode of action (MOA) data and/or elements for PCP-induced carcinogenicity. However, it must be emphasized that since 99% pure PCP is not carcinogenic in animals (with the plausible exclusion of the stop-study results following one year of exposure to 1000 mg/kg discussed above), inferences about potential MOA elements must, by default, be directed at contaminants. The only exception would be if PCP alone caused non-specific high dose toxicity (e.g., cytotoxicity-induced cell proliferation, generation of reactive oxygen species (ROS), etc.)

which obviously did not occur. In addition to the above explanation of the stop-study results in the Chhabra et al. (1999), this would logically explain the lack of carcinogenic effects of 99% PCP at a 2-year dose of 600 mg/kg while a dose of 1000 mg/kg was high enough to induce and/or lead to a variety of cytotoxicity-driven MOA endpoints. In particular, while PCP shows scant evidence of *in vivo* mutagenicity, which would be a necessary precursor for initiation of tumor development from a genotoxic carcinogen, this is not the case if mutations occur only following high-dose toxicity-driven events. For example, as noted in the EPA (2009) IRIS Assessment of Pentachlorophenol there is little *in vivo* evidence of PCP-induced mutagenicity/genotoxicity, i.e., *"…standard mutagenicity assays have produced weak or equivocal evidence for PCP."* Similarly, high dose PCP cytotoxicity-induced events as discussed in the EPA/IRIS (2010) assessment likely involved in animal carcinogenesis, e.g., oxidative stress (ROS), ROS-induced DNA damage/mutation, lipid peroxidation, inhibition of gap junction intracellular communication (GJIC) or chronic inflammation in addition to uncoupling of oxidative phosphorylation could all play an etiological role. None of these issues are addressed in the PCP listing document.

Finally, as discussed below, when analysis/assessment of the human epidemiological data is limited to sub-cohorts exposed only to PCP, there is little evidence of a causal association with NHL. This is particularly the case when the totality of the data is assessed with the well-established causation guidelines (i.e., EPA 2005, NTP 2013, etc.).

With respect to biological plausibility, the causation criteria presumably omitted from the evaluation process, the data do not support a conclusion that PCP should be considered as a known cause of NHL. As summarized in these comments, because it is possible to compare/contrast potential carcinogenic effects from 99% pure PCP from those produced by PCP formulations of approximately 90% purity, the former should be the basis for inferring the carcinogenic of PCP alone. As demonstrated in these comments, neither PCP alone, nor the by-products of synthesis cause cancer in animals. This is supported by little persuasive evidence of *in vivo* mutagenicity of PCP and the documentation that even the cytotoxicity-driven mode of action (MOA) events produced by technical grades of PCP do not occur with PCP alone.

The NTP Listing Document's Assessment of the Epidemiologic Evidence on NHL

The Listing Document concludes, "*There is sufficient evidence for the carcinogenicity of pentachlorophenol from studies in humans*" (p. 77) and further describes the epidemiologic evidence in support of the listing as indicating a "*consistent association between occupational exposure to pentachlorophenol and non-Hodgkin lymphoma that cannot be reasonably explained by chance, bias or confounding*" (p. 77). This is not a convincing or supportable interpretation of the epidemiologic evidence pertaining to non-Hodgkin lymphoma (NHL) for several reasons, including:

- Absence of strong associations in most of the studies and statistical imprecision in several of the key studies
- Lack of consistency among the key studies
- Inadequate evidence of a monotonic exposure-response trend
- The possibility that the positive associations reported reflect residual confounding
- The possibility that some of positive associations reported reflect information bias.

The Listing Document ‘s assessment of the available peer-reviewed epidemiologic studies focused on studies by Demers et al. (2006), Collins et al. (2009), Ruder and Yiin (2011), Kogevinas et al. (1995) and Hardell et al. (1994 and 2002). The Listing Document rated the cohort study by Demers et al. (2006), with additional analyses by Friesen et al. (2007), as providing the strongest evidence of a causal relation between PCP and NHL; the cohort study by Collins et al. (2009) as being supportive; and the studies by Kogevinas et al. (1995), Ruder and Yiin (2011) and by Hardell et al. (1994 and 2002) as providing “more limited” evidence (p. 75).

Demers et al. (2006)

The evidence of a causal relation between PCP and NHL by the Demers et al. (2006) study of Canadian sawmill workers is limited for several reasons. Most important are (1) the striking lack of any excess of NHL mortality or incidence among PCP-exposed workers compared to the general population of British Columbia and (2) the lack of a monotonic exposure-response relation in analyses of NHL rates by level of estimated exposure to PCP within the cohort. The Listing Document asserts that the study of Demers and colleagues demonstrates a “clear” exposure-response relationship for PCP, but this conclusion may not be warranted.

The comparison of the PCP-exposed sawmill cohort’s NHL mortality and incidence rates to those of the general population of the province where sawmills were located indicated a standardized mortality ratio (SMR) of 1.02 and a standardized incidence ratio (SIR) of 0.99. These results, indicating that the rates of sawmill workers were virtually identical to those of the general population, are in marked contrast to results from studies of workers exposed to other established human carcinogens, which typically show an excess of a particular form of cancer among exposed workers compared to a general population at large. In an exposure-response analysis of NHL incidence, rate ratios were 1.0, 1.83. 2.05 and 1.98 (P for trend, 0.02) for categories of exposure years (20-year lag) of <1 (referent category), 1-2, 2-5 and 5+, respectively.

The lack of an overall excess of NHL, coupled with the absence of exposure-response across the exposure categories of 1-2, 2-5 and >5 exposure years, is a pattern of results suggesting (a) that the lowest exposure group (<1 exposure year) may have had a deficit of NHL in comparison with the general population, (b) that none of the higher exposure groups are likely to have had a substantial excess of NHL in comparison with the general population [Note: Contrary to the statement that “*Both external and internal incidence and mortality analyses (by estimated cumulative dermal exposure) were*

analyzed…", the papers by Demers et al. and Friesen et al. did not present external analyses by estimated cumulative exposure.] and (c) that the statistical significance of the exposure-response "trend" was due largely to an unexplained difference in the incidence of NHL between the lowest exposure category and the higher exposure categories that could reflect exposure misclassification or residual confounding. Neither the exposure category-specific rate ratios nor the corresponding trend analyses indicated a "strong" positive association between PCP and NHL.

Demers et al. and Friesen et al. and the Listing Document extensively discussed the possibility of exposure misclassification. However, residual confounding was mentioned mainly as an issue stemming from co-exposure to tetrachlorophenol, and the evidence of confounding by this agent was limited. Other sources of confounding were age and time period. The papers by Demers et al. and Friesen et al. are vague with respect to methods used to control for these factors. The paper by Demers et al. did not provide any details pertaining to the handling of these factors in internal analyses of exposure-response. The paper by Friesen et al. stated, "*We used 10-year calendar period and age categories; to improve precision, categories were combined when necessary to ensure all categories had a minimum of 10 cases*." Although these methods have been appropriate from a statistical perspective, it is not clear if they provided adequate control for age and time period.

Collins et al. (2009)

The study by Collins et al. (2009) investigated mortality among 773 workers with potential exposure to PCP at a Midland (Michigan) plant and evaluated the relation between estimated cumulative exposure to chlorinated dioxins found in PCP and specific causes of deaths, including NHL. Results for NHL were **not** consistent with results of the study by Demers et al. (2006). In contrast to Demers et al., Collins et al. reported an overall excess of NHL mortality in the total cohort (SMR=2.4, 95% CI=1.0-4.7), based on 8 observed and about 3.3 expected deaths, and in the PCP cohort not exposed to trichlorophenol (SMR=2.8, 95% CI=1.1-5.7) (7 observed and about 2.5 expected deaths). Collins et al. observed SMRs of 1.2, 1.2 and 4.5 for low, medium and high cumulative exposure categories, respectively. The SMR in the highest exposure category, based on 4 observed and about 0.9 expected deaths, was statistically significant (95% CI=1.2-11.5), but the data did not display monotonic exposure-response, and there was no statistically significant trend.

Kogevinas et al. (1995)

The nested case-control study of Kogevinas et al. (1995) reported odds ratios of 2.75 (95% CI=0.45-17.00) for any exposure to PCP, based on 3 cases of NHL and 9 controls exposed, and of 4.19 (95% CI=0.59-29.59) for estimated high exposure, based on 3 cases and 5 controls exposed. These results were not statistically significant. The study lacked quantitative estimates of PCP exposure that can be compared with estimates in other studies. Because of the small numbers of cases and controls exposed to PCP, this study was not informative with regard to exposure-response. Although the subjects

were classified as not having known exposure to phenoxy herbicides or other chlorophenols, assessment and control of confounding by other coexposures was not done and would not have been possible due to the small size of the study.

Ruder and Yiin (2011)

The workers included in this cohort study of four plants were exposed to multiple chemicals in addition to PCP: "*One-third of the PCP cohort also worked in departments using tricholorophenol (TCP) or one of its derivatives that were contaminated with 2,3,7,8-TCDD. At the Illinois plant 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufactured elsewhere was esterified and at the Michigan plant, 2,4,5-T, 2,4,5 trichlorophenol (TCP), and 2,4,5-NaTCP were manufactured*."

Also, the workers included in the study overlapped with the workers included in the study by Collins et al. (2009). Specifically, the second largest of the four plants studied by Ruder and Yiin (2011) and referred to as the Midland Michigan plant is the same plant studied by Collins et al., 2009). Ruder and Yiin (2011) included 788 PCP workers from this plant with follow-up through 2005, whereas Collins et al. (2009) included 773 PCP workers from the same plant, followed up through 2004. The precise amount of overlap was not indicated by Ruder and Yiin (2011) but is presumed to be large. The two studies differ markedly in their reports of the number and proportion of the Midland Michigan PCP cohort members probably exposed to TCP: Ruder and Yiin (2011) reported that 675 of 788 PCP workers had been employed in TCP operations at this plant (table 1 of their paper), whereas Collins et al. (2009) reported that only 196 of 773 TCP workers had been employed in TCP operations. No definite explanation is available for this discrepancy.

For the overlapping segment of the cohort (i.e., the Midland Michigan plant), Ruder and Yiin (2011) reported that the SMR for NHL was 2.18 (95% CI=0.94-4.30, 8 observed NHL deaths), similar to the SMR of 2.4 (95% CI=1.0-4.7, 8 observed NHL deaths) reported by Collins et al. (2009). Results for this segment of the cohort studied by Ruder and Yiin (2011) are redundant with results reported by Collins et al. (2009) and do not contribute independently to the epidemiologic evidence pertaining to PCP.

Among workers at one of the plants studied by Ruder and Yiin (2011) (referred to as the Sauget Illinois plant) but not by Collins et al. (2009), the SMR for NHL was 1.81 (95% CI=0.83-3.43, 9 observed NHL deaths). Workers at the latter plant had potential exposure to TCP and 2,3,7,8-TCDD, as well as other chemicals. Ruder and Yiin (2011) did not indicate the number of NHL decedents not exposed to TCP at the Sauget Illinois plant and did not report plant-specific results of analyses for subjects exposed and not exposed to TCP. Among workers at the other two, small plants (referred to as the Tacoma and Wichita plants), who were not exposed to TCP or 2,3,7,8-TCDD, there was no death from NHL, with a very small number expected (number not reported).

This study adds little to the assessment of evidence pertaining to the human carcinogenicity of PCP. The increased SMR for NHL at the Sauget Illinois plant was

based on only nine observed deaths and was not statistically significant. Data from the other two plants were uninformative. The study did not evaluate exposure-response for PCP using quantitative estimates of cumulative exposure to PCP. Instead, the study analyzed SMRs for NHL in relation to days of potential exposure to PCP in the overall cohort and found no positive trend, with the highest SMR observed in the lowest category of days exposed. No data on duration-response were presented separately for the Sauget Illinois plant. The study did not evaluate potential confounding by other chemicals, and the large, unexplained discrepancy between the number of Midland Michigan plant workers classified as exposed to TCP by Ruder and Yiin (2011) and the number so classified by Collins et al. (2009) detracts from the credibility of this research.

[Note: Table 3-4 of the Listing Document reports incorrect cohort sizes for the Sauget Illinois and Midland Michigan plants.]

Hardell et al. (1994, 1999, 2002)

Hardell and colleagues conducted two independent case-control studies of NHL among Swedish men. Both studies contained very limited data on PCP. In these studies, exposure estimation methods were weak, being based largely on self-reported data that were not adequate for deriving quantitative estimates of exposure to agents of interest.

The first study (Hardell et al., 1994), of 105 NHL cases identified during the period 1974-1978 and 335 controls, reported an odds ratio of 8.8 (95% CI=3.4-24.0) for more than one week of continuous exposure or more than one month of total exposure to PCP. This result was based on 15 exposed cases (14.3% of all cases) and 9 exposed controls (2.7% of all controls). Results for lower exposure to PCP were not reported. Thus, no exposure-response data specific to PCP were available from this study. Also, odds ratios for PCP, adjusted for exposure to other agents such as phenoxyacetic acids, or computed for subjects not exposed to such agents, were not reported. The validity of the strong statistical association reported by Hardell et al. (1994) is quite uncertain, given evidence of possible methodological short-comings discussed below and given that PCP exposure could have been quite short, and no details of cases' or controls' exposure histories were provided.

Hardell et al. implied that differential information bias (recall bias) was unlikely because of a high level of agreement between self-reported exposures and employers' records but provided few details of this assessment. The Listing Document concludes that differential information bias may have been avoided in the studies of NHL by Hardell and colleagues because cases and controls were matched on vital status (p. 75). However, such matching does not guarantee absence of recall bias, which remains a possibility in the studies by Hardell and colleagues.

The second study (Hardell and Eriksson, 1999), of 404 NHL cases identified in 1987-1990 and 741 controls, reported an odds ratio for NHL of 1.2 (0.7-1.8) based on 55 exposed cases (13.6% of all cases) and 87 exposed controls (11.7% of all controls). The study did not present analyses of odds ratios by duration or level of exposure to

PCP to assess exposure-response, nor did it present odds ratios for PCP adjusted for exposure to other agents, including phenoxyacetic acetic acids. A subsequent paper (Hardell et al., 2002) pooled data from the study of Hardell and Eriksson (1999) with a small study of hairy cell leukemia that used similar methods (Nordstrom et al, 1998). The pooled study (Hardell et al., 2002) reported odds ratios of 1.40 (0.99-1.98) for NHL combined with hairy cell leukemia and an odds ratio of 2.6 (1.1-6.2) for hairy cell leukemia alone. The Hardell et al. (2002) pooled analysis, like the earlier study by Hardell and Eriksson (1999), did not assess exposure-response for PCP or possible confounding by other exposures.

The results of the studies of NHL by Hardell et al. (1994) and Hardell and Eriksson (1999) are markedly inconsistent. Hardell and Eriksson suggested that the conflicting results of the study of 1974-1978 NHL cases and the study of 1987-1990 cases could be explained by the banning of chlorophenols in Sweden in 1977 and the consequent "lack of late exposure" in the more recent study. This explanation is not consistent with the results of the study by Demers et al. (2006), which reported elevated NHL rate ratios for higher PCP exposure categories in analyses that lagged exposure by 20 years. Such analyses, for example, classified a subject with NHL as unexposed to PCP if exposure began within 20 years of the occurrence of NHL.

One particular aspect of the earlier study (Hardell et al., 1994), which reported a strong association between PCP and NHL, clearly distinguishes it from the second study (Eriksson et al., 1999), which reported essentially a null association This aspect is the much lower proportion of controls classified as exposed to PCP in the former (2.7% of controls) than in the latter (11.7% of controls) study, while proportions of cases classified as exposed to PCP did not vary meaningfully between the two studies (14.3% and 13.6% of cases were exposed in the earlier and later studies, respectively). These patterns suggest that in one or both of the studies of NHL by Hardell and colleagues bias and/or uncontrolled confounding may have affected the results and seriously challenge the validity of these studies.

[Note: participation rates reported on p.41 of the Listing Document appear to be incorrect.]

The NTP Listing Document's Assessment of the Epidemiologic Evidence on Other Cancers

The Listing Document characterizes the epidemiologic evidence pertaining to the relation between PCP and other cancers (multiple myeloma, STS, and cancers of the kidney, liver and lung is even weaker than the evidence for NHL. This conclusion is reasonable. With regard to kidney cancer and multiple myeloma, the Listing Document states (p. 76), "*There was strong evidence for an association between multiple myeloma and moderate evidence for kidney cancer in the most informative … study (Demers et al. 2006), based on statistically significant exposure-response relationships; however, there was little evidence from other studies to support this finding*." This conclusion is not warranted. There was no excess of multiple myeloma when sawmill workers were

compared to the general population. Although trends were positive and statistically significant, as was apparent for NHL, there was no monotonic exposure-response trend across the three high exposure categories for multiple myeloma. In the overall cohort, kidney cancer deaths and incident cases were increased in analyses that compared sawmill workers to the general population. Exposure-response trends were statistically significant in some analyses but were not monotonic.

Summary

The epidemiologic evidence pertaining to the carcinogenicity of PCP in humans is inconclusive and limited. The available studies do not indicate that there is a valid, strong and consistent association between exposure to PCP and NHL or other forms of cancer. Clear evidence of monotonic exposure-response is lacking, and proposed explanations of lack of such trends are speculative at best.

REFERENCES

Baan R, Grosse Y, Straif K, et al. 2009.A review of human carcinogens—Part F: Chemical agents and related occupations. Lancet Oncology 10(12):1143-1144.

Chhabra RS, Maronpot RM, Bucher JR, Haseman JK, Toft JD, Hejtmancik MR. 1999. Toxicology and carcinogenesis studies of pentachlorophenol in rats. Toxicol Sci 48(1): 14-20.

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.

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.

EPA. 2010. Toxicological Review of Pentachlorophenol (CAS No. 87-86-5) in Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-09/004F. Washington, D.C.: U.S. Environmental Protection Agency. 288 pp.

Friesen MC, Davies HW, Teschke K, Ostry AS, Hertzman C, Demers PA. 2007. Impact of the specificity of the exposure metric on exposure-response relationships. Epidemiology 18(1): 88-94.

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. 1999. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer 85(6): 1353-1360.

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.

Hill AB. 1965. The environment and disease: association or causation? Proc R Soc Med 58: 295-300.

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Bueno-de-Mesquita HB, Coggon D, Green L, Johnson E, Littorin M, Lynge E, Marlow DA, Mathews JD, Neuberger M, Benn T, Pannet B, Pearce N, Saracci R. 1995. Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. Epidemiology 6(4): 396-402.

Maronpot, R.R., Zeiger, E., McConnell, E.E, Kolenda-Roberts, H., Wall, H., and Friedman MA. 2009. Induction of tunica vaginalis mesotheliomas in rats by xenobiotics. Crit Rev Toxicol. 2009;39(6):512-37.

NTP. 1980. Bioassay of a Mixture of 1, 2, 3, 6, 7, 8-Hexachlorodibenzo-p-Dioxin and 1, 2, 3, 7, 8, 9-Hexachlorodibenzo-p-Dioxin (Gavage Study) for Possible Carcinogenicity Technical Report Series No. 198. NIH Publication No. 80-1754. Research Triangle Park, NC: National Toxicology Program.

NTP. 1980. Bioassay of a Mixture of 1, 2, 3, 6, 7, 8-Hexachlorodibenzo-p-Dioxin and 1, 2, 3, 7, 8, 9-Hexachlorodibenzo-p-Dioxin (Dermal Study) for Possible Carcinogenicity Technical Report Series No. 202. NIH Publication No. 80-13. Research Triangle Park, NC: National Toxicology Program.

Ruder AM, Yiin JH. 2011. Mortality of US pentachlorophenol production workers through 2005. Chemosphere 83(6): 851-861.

Schwetz B, Quest J, Keeler P, Humiston C, Kociba R 1978. Results of two-year toxicity and reproduction studies on pentachlorophenol in rats. In: Pentachlorophenol Chemistry, Pharmacology and Environmental Toxicology. Edited by KR Rao, Plenum Press, New York and London, pp. 301-309.