CANTOX
HEALTH SCIENCES INTERNATIONAL

July 6, 2009

Office of Environmental Information (OEI) Docket
(Mail Code: 2822T)
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC USA 20460

Re: Draft Toxicological Review of Pentachlorophenol (CAS No. 87-86-5) In Support of Summary Information on the Integrated Risk Information System (IRIS)", Docket ID No. EPA-HQ-ORD-2009-0178

Dear Sir or Madam:

At the request of the Pentachlorophenol Task Force, a Panel of Experts qualified in toxicology and the assessment of carcinogenic risk was requested to review the draft Toxicological Review of Pentachlorophenol (PCP), announced in the Federal Register on May 7, 2009, and comment on the validity of the recommended oral slope factor of 4.0 x 10^{-1} per mg/kg body weight/day for technical PCP. This recommended slope factor is based on the 95% upper confidence limit on the combined risk for male mice that developed liver and/or adrenal gland tumors, and assumes a linear dose response. This recommended oral slope factor is roughly 3 times higher than the existing IRIS slope factor.

The experts that have contributed to these comments include Ian C. Munro, Ph.D., F.A.T.S., FRCPath of Cantox Health Sciences International, Mississauga, ON Bernard A. Schwetz, DVM, Ph.D. retired from the U.S. Department of Health and Human Services, James A. Swenberg, DVM, Ph.D., DACVP of the University of North Carolina Chapel Hill, and Ernest. E. McConnell, DVM, DACVP, DABT of Tox Path Inc, Raleigh, NC..

It is the opinion of the Panel of Experts that the methodology used to derive the oral slope factor is overly conservative and does not incorporate accurately or sufficiently current knowledge on mechanisms of toxicity. As a result, the recommended slope factor exaggerates the risk of cancer in humans associated with PCP exposure. It is the further opinion of the Panel of Experts that the new oral slope factor recommended by EPA for PCP in the draft report be reconsidered and replaced by a non-linear risk assessment.

The basis for the Panel of Experts opinion is explained in detail in the accompanying Technical Comments. In summary, the Panel of Experts notes:

1. The Agency ignored the 1990 recommendation of its own Scientific Advisory Board (SAB) that the tumors in the female mice rather than the male mice should be used to derive the oral slope factor, and in that case only the hemangiomas and hemangiosarcomas observed in the female mice were relevant to known human cancers.

2. Had EPA considered the SAB recommendation, it would have been apparent that the other tumor types observed in the 1989 NTP study either lacked biological or statistical significance, as is explained for each tumor type in the accompanying Technical Comments.

3. Had EPA focused its attention on the hemangiomas and hemangiosarcomas in the female mice, as recommended by the SAB, the recent and extensive literature on the mode of action (MOA) of tumor formation of PCP and its implications for a non-linear dose response relationship would have taken on the proper import.

4. Had EPA considered MOA, as recommended by the Agency's 2005 Cancer Assessment Guidelines, it would not have ignored the 1999 NTP rat study but would have realized that the rat may be a more appropriate animal model for assessing risks of PCP to humans.

Consideration also should be given to the fact that employing overly conservative risk assessment practices serves only to exaggerate health risks of certain chemicals. This has the unintended impact of driving limited public health agency resources to mitigate low priority risks (*i.e.*, from presumed weak human carcinogens) instead of higher priority risks (*i.e.*, from DNA-reactive chemicals known to cause cancer) of greater concern to health of the public.

The Panel respectfully requests that the Agency carefully consider the points made and the information presented in the accompanying Technical Comments and that these materials be provided to the external peer reviewers that have been convened to review the draft IRIS report.

Respectfully submitted,
[Redacted]

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Technical Comments on:

"Draft Toxicological Review of Pentachlorophenol (CAS No. 87-86-5) In Support of Summary Information on the Integrated Risk Information System (IRIS)"

Docket ID No. EPA-HQ-ORD-2009-0178

Prepared for: Office of Environmental Information (OEI)
Docket (Mail Code: 2822T),
U.S. Environmental Protection Agency
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Washington, DC 20460

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July 6, 2009

Technical Comments on: "Draft Toxicological Review of Pentachlorophenol (CAS No. 87-86-5) In Support of Summary Information on the Integrated Risk Information System (IRIS)"

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Technical Comments on: "Draft Toxicological Review of Pentachlorophenol (CAS No. 87-86-5) In Support of Summary Information on the Integrated Risk Information System (IRIS)"

1.0 INTRODUCTION

At the request of the Pentachlorophenol Task Force, a Panel of Experts qualified in toxicology and the assessment of carcinogenic risk was requested to review the draft Toxicological Review of Pentachlorophenol (PCP), announced in the Federal Register on May 7, 2009, and comment on the validity of the oral slope factor recommended by the U.S. Environmental Protection Agency (EPA) for human risk assessment. The experts that have contributed to these comments include Ian C. Munro, Ph.D., F.A.T.S., FRCPath of Cantox Health Sciences International, Mississauga, ON, Bernard A. Schwetz, DVM, Ph.D. retired from the U.S. Department of Health and Human Services, James A. Swenberg, DVM, Ph.D., DACVP of the University of North Carolina Chapel Hill, and Ernest. E. McConnell, DVM, DACVP, DABT of Tox Path Inc., Raleigh, NC.

It is the opinion of the panel that the methodology used to derive the oral slope factor of 4×10^{-1} $(mg/kg\text{-}day)^{-1}$ is overly conservative and does not incorporate accurately or sufficiently current knowledge on mechanisms of toxicity. As a result, the recommended slope factor exaggerates the risk of cancer in humans associated with PCP exposure.

This opinion is based on the following factors, which are discussed in greater detail in the sections that follow:

- The questionable relevance of the male mouse tumors for human risk assessment;
- The question of the suitability of the mouse model as compared to the rat;
- The inappropriateness of combining different tumor types;
- Recent research on oxidative stress supporting the proposition that the mechanism of PCP carcinogenicity does not exhibit a linear dose response relationship, especially in the liver;
- The conservatism of EPA's methods;
- A lack of human data to corroborate the conservatism of EPA's proposed slope factor.

The draft review of the toxicity of PCP by EPA (2009) raises issues of responsiveness to recommendations of outside experts and credibility of the Agency. In preparing the current draft

risk assessment and the previous evaluation, EPA ignored the advice of its Science Advisory Board (SAB, 1990) on the relevance and irrelevance of tumors in male and female mice. EPA's SAB had previously made recommendations about the cancer risk assessment of PCP which were acknowledged by the Agency in a previous risk assessment but seem to be ignored in the current draft. When a federal regulatory agency publishes a new, stricter risk assessment on a chemical under its jurisdiction, it is usually because there are new toxicological data that establish the need for a revised risk assessment, or a consensus has been reached in the field on a more appropriate way to conduct a risk assessment on existing toxicological data. It does not appear that either of these factors has triggered the need for a new cancer risk assessment on PCP. Clearly, this raises the question of the justification for re-analyzing the same data on PCP which was already carefully considered by the SAB apparently in order to derive an elevated potency factor.

Ignoring the recommendations of its scientific advisers should not be done lightly by any regulatory agency. It discredits the advice of the agency's own federal advisory committee and can affect the willingness of the federal advisory committee to take positions contrary to what the agency may want to hear. Some of the rapport between the committee and the agency is lost making it harder to recruit good committee members in the future when an agency doesn't follow the recommendations of its advisers without providing a sound rationale. Both of these factors, conducting a new risk assessment for no apparent reason, and ignoring the recommendations of the SAB, raise serious questions about EPA's objectives and procedures for conducting this review and cancer risk assessment on PCP. At a minimum, there should be clear documentation for why the agency discounted the advice of the SAB and why they thought they needed a new slope factor.

Thus, the panel cautions that disregarding available expert advice can affect the credibility of the EPA risk assessment process.

2.0 ANIMAL DATA

2.1 Summary of Mouse Studies

Two year dietary studies in the B6C3F1 mouse of a technical grade PCP composite (purity 90.4%) and PCP, Dowicide EC-7 (purity 91%) were conducted by the National Toxicology Program (NTP, 1989). While the concentration of PCP was comparable, Dowicide EC-7 was confirmed by analysis to contain lower levels of dioxins and furans than the technical PCP. Results of the studies were published in 1991 (McConnell *et al.*, 1991). Each of the treatment groups included 50 male and 50 female mice while 2 additional groups of 35 males and 35 females per group were fed the control diet (although not all treatment or control animals in each group were included in determining tumor incidence). It was noted that the survival of the male control group for the technical PCP was low, with only 12 of 35 animals surviving to the

end of the study, although survival of control animals in the Dowicide EC-7 was not at issue (25/35). The results of these studies formed the basis of the oral slope factors derived by EPA in the earlier 1991 IRIS assessment, and in the current 2009 draft. The tumor incidence findings are summarized in Table 2.1-1.

Table 2.1-1 Summary of Tumor Incidence Data from Two-Year Feeding Studies with Two Grades of Pentachlorophenol in B6C3F1 Mice

	Technical Pentachlorophenol			Dowicide EC-7			
ppm diet	0	100	200	0	100	200	600
mg/kg bw/day – males	0	18	35	0	18	37	118
mg/kg bw/day – females	0	17	35	0	17	34	114
Survival – males	12/35 (34%)	24/50 (48%)	22/50 (44%)	25/35 (71%)	28/50 (56%)	29/50 (58%)	35/50 (70%)
Survival – females	28/35 (80%)	41/50 (82%)	30/50 (60%)	29/35 (83%)	28/50 (56%)	38/50 (76%)	39/50 (78%)
Tumors Observed in Male Mice							
Hepatocellular Adenomas	5/32 (15.6%)	20/47** (42.5%)	33/48* (68.8%)	5/35 (14.3%)	13/48 (27.1%)	17/48* (35.4%)	32/49** (65.3%)
Hepatocellular Carcinomas	2/32 (6.3%)	10/47 (21.3%)	12/48* (25%)	1/35 (2.9%)	7/48 (14.6%)	7/48 (14.6%)	9/49* (18.4%)
Combined Hepatocellular Adenomas and Carcinomas	7/32 (21.9%)	26/47** (55.3%)	37/48* (77.1%)	6/35 (17.1%)	19/48 (39.6%)	21/48* (43.8%)	34/49* (69.4%)
Adrenal Medulla Pheochromocytoma	0/31 (0%)	10/45** (22.2)	23/45** (51.1)	0/34 (0%)	4/48 8.3	21/48** 43.8	44/49** 89.8
Adrenal Medulla malignant pheochromocytoma	0/31 (0%)	0/45 (0%)	0/45 (0%)	1/34 (2.9%)	0/48 (0%)	0/48 (0%)	3/49 (6.1%)
Combined Adrenal Medulla pheochromocytoma and malignant pheochromocytoma	0/31 (0%)	10/45** (22.2%)	23/45** (51.1%)	1/34 (2.9%)	4/48 (8.3%)	21/48** (43.8%)	45/49** (91.8%)
Tumors Observed in Female Mice							
Hepatocellular Adenomas	3/33 (9.1%)	8/49 (16.3%)	8/50 (16.0%)	1/34 (2.9%)	3/50 (6.0%)	6/49 (6.1%)	30/48** (62.5%)
Hepatocellular Carcinomas	0/33 (0%)	1/49 (2.0%)	1/50 (2.0%)	0/34 (0%)	1/50 (2.0%)	0/49 (0%)	2/48 (4.2%)
Combined Hepatocellular Adenomas and Carcinomas	3/33 (9.1%)	9/49 (18.4%)	9/50 (18.0%)	1/34 (2.9%)	4/50 (8.0%)	6/49 (12.2%)	31/48** (64.6%)
Adrenal Medulla Pheochromocytoma	0/33 (0%)	2/48 (4.2%)	1/49 (2.0%)	0/35 (0%)	1/49 (2.0%)	2/46 (4.3%)	38/49** (77.6%)
Adrenal Medulla Malignant Pheochromocytoma	2/33 (6.1%)	0/48 (0%)	0/49 (0%)	0/35 (0%)	1/49 (2.0%)	0/46 (0%)	1/49 (2.0%)

Table 2.1-1 Summary of Tumor Incidence Data from Two-Year Feeding Studies with Two Grades of Pentachlorophenol in B6C3F1 Mice

	Technical Pentachlorophenol			Dowicide EC-7			
Combined Adrenal Medulla Pheochromocytoma and Malignant Pheochromocytoma	2/33 (6.1%)	2/48 (4.2%)	1/49 (2.0%)	0/35 (0%)	2/49 (4.1%)	2/46 (4.3%)	38/49** (77.6%)
Vascular Hemangioma	0/35 (0%)	0/50 (0%)	0/50 (0%)	0/35 (0%)	0/50 (0%)	0/50 (0%)	1/49 (2.0%)
Vascular Hemangiosarcoma	0/35 (0%)	3/50 (6.0%)	6/50* (12.0%)	0/35 (0%)	1/50 (2.0%)	3/50 (6.0%)	8/49* (16.3%)
Combined Vascular Hemangioma and Hemangiosarcoma	0/35 (0%)	3/50 (6.0%)	6/50* (12.0%)	0/35 (0%)	1/50 (2.0%)	3/50 (6.0%)	9/49* (18.4%)

Source – adapted from EPA draft dossier (2009) and McConnell *et al.* (1991); note that the number of hemangiosarcomas reported for the high dose females administered Dowicide EC-7 differs between sources; according to the detailed NTP (1989) report, the McConnell publication information is correct
* $p < 0.05$ *vs.* controls; ** $p < 0.01$ *vs.* controls; bw = body weight

Signs of liver toxicity that accompanied the findings of increased hepatocellular tumor incidences in mice included clear cell foci, multifocal proliferation of hematopoietic cells, diffuse chronic active inflammation, multifocal pigmentation, acute diffuse necrosis, diffuse cytomegaly, and bile duct hyperplasia.

The historical control data for B6C3F1 mice, for the tumor types seen in the PCP studies, are based on 19 dietary cancer bioassay studies conducted by the NTP (1999a), and are summarized in the Table 2.1-2. These data are from studies conducted with the NIH 7 diet, which was used in the PCP studies.

Table 2.1-2 Summary of Historical Tumor Incidence Data from 19 Two-Year Feeding Studies in B6C3F1 Mice						
Tumor Type	Male Mice			Female Mice		
	Total	Mean (sd)	Range	Total	Mean (sd)	Range
Hepatocellular Adenomas	363/950	38.2%(10.7)	9/50 - 30/50 (18 to 60%)	218/951	22.9%(9.9)	6/50 - 25/50 (12 to 50%)
Hepatocellular Carcinomas	194/950	20.4%(6.7)	5/50 - 20/50 (10 to 40%)	104/951	10.9%(4.6)	2/49 - 10/50 (4.1 to 20%)
Hepatocellular Adenomas and Carcinomas	493/950	51.9%(7.9)	20/50 - 34/50 (40 to 68%)	292/951	30.6%(10.2)	6/49 - 28/50 (12.2 to 56%)
Adrenal Medulla Pheochromocytoma	6/944	0.6%(1.1)	0/49 - 2/52 (0 to 3.8%)	7/944	0.7%(1.2)	0/49 - 2/50 (0 to 4%)
Adrenal Medulla Malignant Pheochromocytoma	1/944	0.1%(0.5)	0/49 - 1/50 (0 to 2%)	2/944	0.2%(0.6)	0/49 - 1/50 (0 to 2%)
Adrenal Medulla Benign and Malignant Pheochromocytoma	7/944	0.7%(1.2)	0/49 - 2/52 (0 to 3.8%)	9/944	0.9%(1.2)	0/49 - 2/50 (0 to 4%)
Hemangioma	7/952	0.7%(1.2)	0/50 - 2/50 (0 to 4%)	14/953	1.5%(1.7)	0/50 - 2/49 (0 to 4.1%)
Hemangiosarcoma	53/952	5.6%(3.5)	1/50 - 7/50 (2 to 14%)	31/953	3.3%(2.4)	0/52 - 4/50 (0 to 8%)
Hemangioma and Hemangiosarcoma	60/952	6.3%(4.2)	1/50 - 9/50 (2 to 18%)	44/953	4.6%(2.3)	1/52 - 4/49 (1.9 to 8.2%)

Source: NTP, 1999a http://ntp.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/m_orlfd.txt

2.2 Summary of Rat Studies

A dietary carcinogenicity study of PCP also was reported in F344 rats by the NTP (1999b) (Chhabra *et al.*, 1999). In this study, rats (50/sex/group) were administered diets containing 200, 400, or 600 ppm PCP (or approximately 10, 20, or 30 mg/kg body weight/day) for 105 weeks. This study also included a "stop-exposure" (recovery) component in which additional groups of rats (60/sex/group) were administered 0 or 1,000 ppm PCP in the diet (approximately 60 mg/kg body weight/day) for 52 weeks, and undosed feed for the remainder of the 2-year study, with the exception of 10 male and 10 female rats/group, which were evaluated at 7 months. The purity of the PCP used in this study was approximately 99%, which is higher than that of the mouse studies.

No PCP-related increases in neoplastic lesions were observed in males or females administered PCP for the full 2-year study period. The incidence of malignant mesotheliomas originating from the tunica vaginalis of the testes was significantly increased in the "stop-exposure" males (9/50 at 1,000 ppm *versus* 1/50 for controls). This tumor type was not observed in the male mice. The historical incidence rate for this tumor type was reported to be

0 to 8%. Also, the incidence of nasal squamous cell carcinoma in the "stop exposure" males exceeded the historical control range (0 to 4%), but the increase was not statistically significant compared to controls (5/50 at 1,000 ppm *versus* 1/50 for controls).

The tumors observed in the rat study are of doubtful significance and relevance to human risk assessment given that the incidence of tumors was significantly increased in the high-dose "stop-exposure" group males only, and not at the 600 ppm dose males, following 2 years of exposure, despite the fact that the 2-year exposed rats had a greater total life-time dose. Also, these tumors were not increased in the male mice.

2.3 Discussion of Mechanism of Carcinogenic Action

2.3.1 Liver Tumors in Mice

Liver adenomas and carcinomas are the most commonly observed neoplasm in male B6C3F1 mice and the second most commonly occurring tumor in female mice (Eaton and Gilbert, 2008). In addition, it is noteworthy that the incidences of liver adenomas/carcinomas in the PCP study concurrent controls were lower than the historical control incidence data which may be as high as 68% for males and 56% for females, with average incidences of 51.9 and 30.6%, respectively. In fact, the incidence data for the concurrent control groups in the technical PCP and Dowicide EC-7 studies are below the lower end of the historical incidence range, of 40 and 12.2% for males and females, respectively.

Numerous studies on a possible mode of action for the liver tumors observed in the pentachlorophenol studies in mice were reviewed by EPA (2009) but dismissed as being inconclusive. However, chronic oxidative stress leading to generation of reactive oxygen species and liver toxicity are strongly supported by the most current data as the mechanism for tumor induction. Numerous publications have documented the induction of oxidative stress by PCP, with most of them cited in the EPA (2009) document. In addition, a new paper was just published in Chemical Research in Toxicology (Zhu and Shan, 2009) that demonstrates the mode of action for formation of the hydroxyl radical. These papers have shown dose-related increases in apurinic and apyrimidinic (AP or abasic) sites, 8-OHdG and single-strand breaks in DNA. While such lesions represent the most common endogenous forms of DNA damage, they were further increased at high exposures to PCP and its metabolites. As such, the oxidative stress mode of action only applies to high exposures. No increases are expected at low exposures, so that a biologically-based risk assessment for PCP is expected to be non-linear (Swenberg, *et al.*, 2008). Furthermore, species differences can be explained by differences in the rate of glutathione depletion. Conjugation of reactive oxygen species with glutathione, as catalyzed endogenously by glutathione transferases, is part of a protective mechanism against toxicity induced by reactive oxygen species (Parkinson and Ogilvie, 2008). Thus, the potential for liver toxicity resulting from oxidative stress increases as glutathione is depleted. Studies with acetaminophen have demonstrated that, following intraperitoneal administration of 300 mg/kg

body weight, glutathione was rapidly depleted in the mouse by about 80% after 90 minutes, with only 10% being depleted in the rat at the same dose and after the same amount of time had elapsed (Davis *et al.*, 1974). In comparison, humans were reported to be relatively resistant to hepatotoxic effects of acetaminophen (Tee *et al.*, 1987). Furthermore, in evaluating the mode of action for styrene effects on the lung, glutathione depletion was reported to be more prominent in mice than in rats and not expected to occur in humans (EC, 2004). Humans are not anticipated to be susceptible to glutathione depletion at current exposures to PCP. Thus, neither oxidative stress, nor glutathione depletion is likely to occur at the levels to which humans are exposed to PCP.

Finally, the much lower incidences of liver tumors with Dowicide EC-7 compared to the same dose of technical PCP, is evidence that the dioxin and furan contaminants, which are higher in the technical PCP, than the Dowicide EC-7, may contribute greatly to the increased incidence of liver tumors. These contaminants also are present in Dowicide EC-7, although at lower concentrations than the technical PCP; however, it is possible that the effects of Dowicide EC-7 could still be related to the contaminants and not PCP alone.

2.3.2 Adrenal Tumors in Mice

The incidence of malignant pheochromocytomas was not significantly increased in the mouse studies at any dose in either males or females. The benign tumors are not considered relevant to humans. There is no epidemiology evidence to support that these lesions can be induced in humans under any conditions (Elmore *et al.*, 2009). Thus, in contrast to pathologies of other target organs in rats and mice induced by chemical exposures, there is no evidence to suggest that these proliferative lesions, which are the major adrenal medullary pathology findings in rodent studies, have any relevance to assessment of human risk (Elmore *et al.*, 2009). This opinion of lack of relevance is supported by the SAB statement.

2.3.3 Vascular Tumors in Mice

The hemangiomas and hemangiosarcomas observed in female mice were reported by the SAB to be "morphologically related to known fatal human cancers that are induced by xenobiotics". However, since the time of that report, much research has been conducted on vinyl chloride (VC), the most studied human carcinogen that causes hemangiosarcoma. VC is metabolized to chloroethylene oxide, a potent mutagen that induces 7-oxoethylguanine and 3 etheno DNA adducts (Swenberg *et al.*, 1992). All 4 of these adducts have now been shown to be induced by oxidative stress (Mutlu *et al.*, 2008a,b, Gao *et al*, 2009). Thus, this mode of action (MOA) is entirely compatible with the formation of hemangiosarcomas in mice chronically exposed to PCP. Again, it will have a non-linear dose-response.

2.3.4 Peritoneal Tumors in Rats

An increased incidence of mesotheliomas of the peritoneal cavity originating from the tunica vaginalis of the testes in male rats was observed in the "stop-exposure" component of the rat study, arising after 1-year exposure to 1,000 ppm PCP (~60 mg/kg body weight/day), but not in the male mice. These tumors also were not significantly increased in the full 2-year exposure study in male rats administered PCP in the diet at concentrations up to 600 ppm (~30 mg/kg body weight/day). Mesotheliomas were reported to be the most common spontaneous neoplasm of the peritoneal cavity of male F344/N rats with a historical incidence of 0 to 8% (Chhabra *et al.*, 1999). However, the tumor incidence in the stop-exposure, at 18%, exceeded the historical range. Peritoneal mesotheliomas are frequently associated with oxidative stress, thus that MOA is again the most likely operative mechanism for tumor induction. In addition to oxidative stress, another non-genotoxic mechanism contributing to this tumor type includes hormonal imbalance brought about by perturbations of the endocrine system, which is associated with the formation of Leydig tumors of the testes which occurs spontaneously at a very high incidence rate in F344/N rats (Haber *et al.*, 2009; Maronpot *et al.*, 2009)

The incidence of nasal squamous cell carcinomas in this rat study also exceeded the historical control range although the increase did not reach statistical significance. Possible explanations proposed by Chhabra *et al.* (1999) for these tumors included direct contact of the nasal mucous membrane to PCP vapor during feeding or exposure to PCP-containing feed dust. Oxidative damage to mesothelial cells of the tunica vaginalis was proposed by the authors as a possible mechanism to explain the mesotheliomas. Two points are important: the incidence of nasal squamous cell carcinomas in the full two-year study was not increased up to 600 ppm in the diet, raising doubt about the significance of the increase seen in the stop exposure study, and most importantly, these tumors are not relevant to humans at current levels of exposure to PCP. For the general U.S. population, exposures to PCP, estimated based on a clearance concept (wherein the net daily intake is assumed to equal the clearance times average steady state plasma concentration) were reported to range from 0.0123 to 0.157 mg/day (~0.00018 to 0.0022 mg/kg body weight/day) (Reigner *et al.*, 1992). Exposure estimates for occupationally exposed individuals ranged widely from 0.035 to 24 mg/day (~0.00050 to 0.34 mg/kg body weight/day) (Reigner *et al.*, 1992).

2.4 Discussion of Species Differences

In risk assessment, exposure limits for humans are typically based on data for the most sensitive species when data on the mechanism of action are absent, which is not the case for pentachlorophenol. However, care is usually taken to ensure the tumor type selected for the basis of an exposure limit is relevant to human health. Liver adenomas/carcinomas are the most common spontaneously occurring tumors in male mice and the second most common in female mice (Eaton and Gilbert, 2008). Furthermore, given the variability of spontaneously

occurring tumors in rodents, it is advised that results for concurrent controls be compared to historic controls, to identify potential "false positives" (Eaton and Gilbert, 2008). The SAB also noted that the unusual sensitivity of the B6C3F1 mouse to hepatocarcinogenesis suggests that the liver of this species may be "primed for tumor development".

Data supporting the greater sensitivity of the male mice are ignored for the purpose of EPA's draft 2009 IRIS assessment. The historical control data show that between 40 and 68% (mean, 51.9%) of B6C3F1 male mice develop hepatocellular adenomas/carcinomas by 2 years. In fact, the incidences of these tumors in the concurrent control groups in the PCP studies are surprisingly low at 21.9% (technical PCP) and 17.1% (Dowicide EC-7). While the reason for this is uncertain, the low survival of control animals in the technical PCP study (12/35) and smaller number of animals in the control groups (35 rather than 50) may have been contributing factors. It also is noteworthy that only the high dose groups of male mice in the PCP studies were outside the historical control range, with the Dowicide EC-7 only marginally increased at 69.4%. The liver tumor incidences for technical PCP, compared to the same doses of Dowicide EC-7, were higher (55.3% *versus* 39.6%, at 100 ppm, respectively, and 77.1% *versus* 43.8% at 200 ppm, respectively). It is probable that the higher levels of dioxins and furans (which are recognized liver toxicants) in technical PCP contributed to this difference. This same relationship held true for the liver tumor results in the female mice (18.4 *versus* 8.0% at 100 ppm, and 18.0 *versus* 12.2% at 200 ppm, for the technical PCP and Dowicide EC-7, respectively). Also, similarly to the male mice, the incidence of hepatocellular adenomas/ carcinomas for the concurrent control female mice at 9.1 and 2.9% were below the historical control ranges for female mice of 12.2 to 56% (mean 30.6%). Among the female treatment groups, only the liver tumor incidence at the highest dose of Dowicide EC-7 (600 ppm) was outside the historical control range at 64.6%.

In contrast to male B6C3F1 mice, in which liver tumors are the most frequently observed neoplasm, the occurrence of liver adenomas/carcinomas in male F344 rats is only the 11th most frequently occurring tumor in the F344 strain (Eaton and Gilbert, 2008). Hence, an increased incidence of liver tumors in the 2-year rat study would have provided more convincing evidence that PCP is hepatocarcinogenic. Furthermore, as the test article for the rat study was 99% pure compared to ~91% in the mouse PCP studies, the possible confounding effect of dioxins/furans would not have been as great an issue. Importantly, an increased incidence of this tumor type was not observed in the 2-year NTP (1999b) rat study.

The hemangiomas/hemangiocarcinomas are the 5th most common spontaneously occurring tumor for female B6C3F1 mice. Unlike the liver tumors, the increased incidence of this tumor type at the high dose is statistically significant compared to concurrent controls and approximately twice the historical control incidence at the highest dose of Dowicide EC-7. However, the incidence of this tumor type was only increased in female mice and not in male mice or in either sex of rats, and the incidence was only statistically significantly increased at the

highest doses tested (200 mg/kg body weight for technical PCP, and at 600 mg/kg body weight/day, but not 200 mg/kg body weight/day, for Dowicide EC-7).

That said, as glutathione conjugation is an important detoxication mechanism of reactive oxygen species, the greater tendency for glutathione depletion to occur with the mouse than the rat and human, as demonstrated in the liver and the lung, suggests that the rat may be a more appropriate animal model for assessing risks of PCP to humans. Also, the purity of the PCP used in the rat study was 99% compared to only 90.4 to 91% in the mouse studies.

4.0 CALCULATION OF ORAL SLOPE FACTOR

4.1 Science Advisory Board (SAB) Opinion

The NTP (1989) mouse studies were evaluated by the SAB in 1990 at the request of the Agency. Based on the results of the mouse studies, the SAB at that time recommended that PCP be classified as a probable human carcinogen (B2). With respect to the individual tumor types observed to be increased in the treated mice, the SAB concluded the following:

- The increased incidence of hepatocellular carcinomas and adenomas was considered to be a "valid indicator of oncogenicity"; however, given the unusual sensitivity of the B6C3F1 mouse to hepatocarcinogenesis, this tumor type should be considered "less important than the appearance of hemangiosarcomas". Also, the concurrent hepatoxicity observed may have "played a key role in the formation of the tumors".

- The increased incidence of pheochromocytomas was considered to be treatment related; however, as the tumors were benign rather than malignant, the relevance of these tumors to human cancer was considered questionable.

- The hemangiomas and hemangiosarcomas observed in female mice were considered by the SAB to be more likely than the other tumor types to be similar to human cancers and thus were recommended for use in quantifying the cancer risk to humans (*i.e.*, derivation of the slope factor). However, it is now known that they are also increased by oxidative stress.

- The use of a toxicity equivalence factor for liver tumors, due to the presence of dioxins, furans, and hexachlorobenzene impurities in the PCP, should not be used.

- It was reasonable to average slope factors across grades of PCP as tested but "averaging across tumor types and genders should not be done".

As the current EPA evaluation is based on the same mouse studies, the opinions and recommendations of the SAB still remain valid and should not be ignored.

4.2 Previous IRIS Evaluation

The previous IRIS evaluation (EPA, 1991) based the quantitative risk assessment on the tumor incidence data for the female mice but, contrary to the recommendations of the SAB, the slope factor was determined from pooled incidence data for female animals with any of the three tumor types (hepatocellular adenoma/carcinoma, pheochromocytoma/malignant pheochromocytoma, hemangioma/hemangiosarcoma). Thus, the tumor types were effectively added (less double counting of animals with more than one of the tumor types) regardless of having different cellular origins and affecting different organs. The resultant oral slope factor of 1.2E-1 per mg/kg body weight/day was the geometric mean of the slope factors determined individually for the technical PCP and Dowicide EC-7 test agents.

4.3 Current Draft EPA Evaluation

In the current draft assessment of PCP, the recommended oral slope factor is based on male mice and derived by combining adrenal and liver tumors, thus ignoring all of the recommendations of the SAB. The draft IRIS assessment also ignores the 2005 EPA Cancer Risk Assessment Guidelines, which state that the preferred method for risk assessment is using a biologically-based model that incorporates MOA considerations. In the case of PCP, an MOA of oxidative stress has been shown in numerous studies, including the NTP (1999b) cancer bioassay in rats (Lin *et al.*, 2002). Oxidative DNA damage is the most common endogenous DNA damage present in all cells. Thus, there is always a background level of endogenous DNA adducts and abasic sites that contribute to spontaneous mutations and neoplasia (Swenberg *et al.*, 2008). Exposure to high doses of PCP results in the formation of additional identical adducts and an increase in neoplasia. However, such increases will not be linear. Therefore, risk assessments for such agents should use a non-linear approach, not slope factors.

The new recommended slope factor also is based on the 95% upper confidence limit on the combined risk for male mice that developed liver and/or adrenal gland tumors. Specifically, the oral slope factor recommended for the technical PCP is 4.0×10^{-1} per mg/kg body weight/day with the stipulation that it not be used with exposures greater than 0.3 mg/kg body weight/day which is the point of departure for the site with the greatest response for technical PCP-exposed male mice. This slope factor also is recommended for use with pure PCP, in spite of the fact that EC-7 was indicated to have lower levels of dioxins and furans. The slope factor recommended by EPA for use with EC-7 is 2.0×10^{-1} per mg/kg body weight/day if exposures are less than 1 mg/kg body weight per day, which is the point of departure for the site with the greatest response for EC-7 exposed male mice. At doses above the point of departure, EPA is concerned that the slope factor may not approximate the observed dose-response relationship.

In addition to the above oral slope factor calculations, EPA also calculated slope factors for the individual tumors and for combined tumors in females. Furthermore, while possible mechanisms of actions were discussed, EPA concluded that there is still uncertainty in this area

and thus chose to apply all conservative defaults of the risk assessment methodology. This included using the most sensitive species (the male mouse), but assuming that humans are more sensitive. These conservative assumptions were made in spite of the overwhelming evidence indicating that liver tumors are very common in the mouse and occurred in the presence of extensive liver toxicity. Furthermore, the historical control data for the NIH 7 diet demonstrated a higher incidence of these tumors than the concurrent control groups in the PCP studies. In addition, the benign tumor response in the mouse liver and adrenal are more reflective of an epigenetic or non-genotoxic mode of action, probably in response to a sustained increased in cellular turnover in the liver and a hormonal challenge, and the hemangiomas/ hemangosarcomas, if a direct response to PCP, may be accentuated by the contaminants and oxidative stress that occurs at high exposures.

The oral slope factors evaluated for the individual tumor data are summarized in Table 4.3-1.

Table 4.3-1 Summary of Oral Slope Factors for Tumors Observed in Mice Cancer Bioassay Studies

	Male Mice Slope Factor $(mg/kg/day)^{-1}$		Female Mice Slope Factor $(mg/kg/day)^{-1}$	
	Technical Pcp	**Dowicide Ec-7**	**Technical Pcp**	**Dowicide Ec-7**
Hepatocellular Adenomas/Carcinomas	2.9×10^{-1}	8.7×10^{-2}	5.6×10^{-2}	4.0×10^{-2}
Adrenal Medulla Pheochromocytoma/Malignant Pheochromocytoma	1.5×10^{-1}	1.1×10^{-1}	NA	2.2×10^{-2}
Hemangioma/Hemangiosarcoma	NA	NA	4.0×10^{-2}	1.7×10^{-2}
Hepatocellular Adenomas/Carcinomas or Adrenal Medulla Pheochromocytoma/Malignant Pheochromocytoma	central tendency: 2.9×10^{-1} upper bound: 4.0×10^{-1}	central tendency: 1.1×10^{-1} upper bound: 1.7×10^{-1}	NA	NA
Hepatocellular Adenomas/Carcinomas or Adrenal Medulla Pheochromocytoma/Malignant Hemangioma/Hemangiosarcoma	NA	NA	central tendency: 5.2×10^{-2} upper bound: 8.3×10^{-2}	central tendency: 2.8×10^{-2} upper bound: 4.8×10^{-2}

Source: EPA, 2009; NA = not applicable

5.0 CONCLUSIONS

The purpose of conducting animal studies is to provide data for assessing potential risks to humans. There is no benefit to public health in greatly overestimating such risks to human health. In fact, significantly overestimating risks is contrary to good public health practice. Such conservative practices greatly overstate the risk of exposures to chemicals that are at most presumed weak human carcinogens over those known to be DNA-reactive human carcinogens. Overstated risks drive state and federal resources to maximize low priority risks instead of higher priority risks of greater concern to the health of the public.

We conclude that the robust science base on PCP argues that the new oral slope factor recommended by EPA for PCP in the draft report be reconsidered and replaced by a non-linear risk assessment.

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