**PENTACHLOROPHENOL TASK FORCE**
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**Re: COMMENTS ON PRIORITIZATION: CHEMICALS IDENTIFIED FOR CONSULTATION WITH THE CALIFORNIA DEVELOPMENTAL AND REPRODUCTIVE TOXICANT IDENTIFICATION COMMITTEE, AUGUST 2015**

Dear Ms. Robinson:

These Comments are submitted by the Pentachlorophenol Task Force (PTF) which represents the U.S. and Canadian registrant of pentachlorophenol ("PCP") in response to the Office of Environmental Health Hazard Assessment (OEHHA) document entitled "Prioritization: Chemicals Identified for Consultation with the Developmental and Reproductive Toxicant Identification Committee, August 2015" (hereinafter "OEHHA 2015"), specifically with respect to the proposal to advance PCP to the next stage of the listing process for developmental and reproductive toxicants under California Proposition 65. Integral Consulting, Inc. has separately filed comments on the epidemiologic data identified in the OEHHA 2015 document which demonstrate that PCP should not proceed to the next stage of the listing process because the fundamental epidemiologic data screen requirements are not met. The PTF supports the Integral comments. The Comments below discuss the animal studies summarized in the OEHHA 2015 document.

OEHHA has issued a document entitled "CRITERA FOR RECOMMENDING CHEMICALS FOR LISTING AS 'KNOWN TO THE STATE TO CAUSE REPRODUCTIVE TOXICITY'" (hereafter the "reproductive criteria"). Parts 1 and 2 of the "reproductive criteria" define what is meant by reproductive toxicity, briefly to include reproductive toxicity in both in males and females and also developmental toxicity. Part 3 of the "reproductive criteria" states that the tests must meet at least one of three criteria; A. Sufficient evidence in humans, (B) Limited or suggestive evidence in humans supported by sufficient mammalian data or, (C) Sufficient experimental evidence in mammals, such that extrapolation to humans is appropriate. The rest of the criteria define these three criteria in a conventional manner that would be generally accepted by toxicologists.

The OEHHA 2015 document references nineteen (19) animal studies and one (1) meeting abstract reporting which are purported to identify reproductive or developmental toxicity, as well as three (3) studies reporting no reproductive or developmental toxicity. Twenty (20) related articles and eleven studies without abstracts were identified.

Although we recognize that the OEHHA 2015 document is intended to reflect a screening of the literature without an in-depth review of the cited references we wish to point out that many of the animal studies cited in OEHHA 2015 do not have any real value in contributing to an evaluation of the human reproductive or developmental toxicity risk of PCP. These studies may be divided into the following categories:

(1) ***Studies in non-mammalian species with no quantitative relevance to humans***. These studies may suggest a possible mechanism for toxicity that may be applicable to mammals as well as to other taxonomic families. These studies are designed primarily to reveal a capacity of PCP to cause reproductive or developmental effects in wildlife species, not humans. These studies are explicitly excluded by the "reproductive criteria" See 3. B and C. The following studies cited in OEHHA 2015 fall into this category.

**Group (1) Non-mammalian**

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|---|---|---|---|
| **Tokumoto et al.** | **(2005)** | Zebra fish oocytes | (page 37 of OEHHA 2015) |
| **Tokumoto et al.** | **(2008)** | Zebra fish oocytes | (page 35 of OEHHA 2015) |
| **Xu et al.** | **(2014)** | Zebra fish oocytes | (page 28 of OEHHA 2015) |
| **Chen et al.** | **(2014)** | Daphnia magna | (page 32 of OEHHA 2015) |
| **Ikuno et al.** | **(2008)** | Daphnia magna | (page 35 of OEHHA 2015) |
| **Buono et al.** | **(2012)** | Sea urchin | (page 33 of OEHHA 2015) |
| **Song et al.** | **(2007)** | Fresh water snail larvae. (Galba pervia); non-biting midge (Chironomus plumousus), and worm (Tubifix sinicus) | (page 36 of OEHHA 2015) |
| **Crovau and Moia** | **(2006)** | Springtail (Folsomia candida) | (page 36 of OEHHA 2015) |
| **Zha et al.** | **(2006)** | Japanese Medaka (Oryzias latipes) | (page 37 of OEHHA 2015) |
| **Zheng et al.** | **(2005)** | Brachydanio rerio embryos | (pages 29-30 OEHHA 2015) |

Similarly, **Maenpaa et al. (2004)** (page 39 of OEHHA 2015) examined PCP bioaccumulation and effect on heat production on salmon eggs at different stages of development. This study is of limited utility for extrapolation to humans having looked only at lake salmon eggs and only with regard to PCP bioaccumulation, partitioning (eggshell, embryo, yolk), and mechanistic information (heat dissipation by uncoupling oxidative phosphorylation).

**Anderson et al. (1994)** (page 41 of OEHHA 2015) expressly states that tests developed primarily for human health protection do not reliably predict the effects of toxic substances on aquatic life. The converse of that proposition is also true. The tests developed for assessing aquatic life do not reliably predict the effects of toxic substances in humans due to major differences in metabolic activation pathways among organisms. This study used sea urchin (Strongylocentrotus purpuratus) gametes and embryos, which would be of limited utility for humans. The exposure durations were too short, 2 hours and 48 hours, to have meaningful indications for human exposure.

**van Gestel et al. (1992)** (page 42 of OEHHA 2015) used earthworm, which is of limited relevance to humans. The PCP used was only 95% pure and the rest of the composition was not disclosed. The doses tested were sublethal and lethal, which is not the case for the human exposure to PCP of interest. PCP at

the highest concentration tested (60 mg/kg) reduced cocoon production, but stimulated earthworm growth. Such effects appeared to be contradictory and were not explained in the article.

**Stephenson et al. (1991)** (page 42 of OEHHA 2015) used Daphnia magna, which is of limited utility for extrapolation to humans. The study was conducted prior to the publication of ASTM and EPA protocols and it departed from the protocol in several areas. The most significant departure from the protocol criteria was that the study used Daphnia magna of 48-h old and the protocol required neonates (≤ 24 h). The article argued that exposure to PCP one day earlier would probably not have affected the results of this study since only two broods were aborted in adult daphnids just prior to death. However, it is conceivable that one day would make a significant difference especially in the study for development, given the short lifespan of Daphnia (a maximum of about two months).

**van Gestel et al. (1989)** (pages 42-44 of OEHHA 2015) used earthworm, which is of limited relevance to humans. The objective of this study was to test a standardized way to study the effects of chemicals on earthworm reproduction. The purity of PCP was not clearly addressed. Effects occurred at the highest concentration tested (100 mg/kg). No dose response was observed.

**Holcombe et al. (1982)** (page 44 of OEHHA 2015) used fathead minnows, which is of limited relevance to humans. The purity of the PCP was low, only 90%±14% and the rest of composition or possible contaminants were not disclosed. There were a higher number of nonviable eggs in the batch of embryos used to start the PCP test than that in the control, which would impact the integrity of the study and make the result questionable.

**Nagler et al. (1986)** (pages 44-45 of OEHHA 2015) used rainbow trout, which is of limited relevance to humans. The doses tested were sublethal, which is not the case for the human exposure to the PCP of interest. The exposure duration was 18 days, which may be insufficient for the detection of reproductive effects. There was no dose-response and the mid-dose had a slightly more severe effect than the high-dose, which is not explained.

**Dave G. (1984)** (page 45 of OEHHA 2015) used embryos and larvae of zebrafish, which is of limited relevance to humans. The objective of the study was to find a laboratory procedure for determination of the effect of pH on the toxicity of various chemicals. PCP was selected as a reference or positive control due to its known pH-toxicity interaction in fish. No details are provided on purity of the PCP.

**Dominguez et al. (1984)** (page 45 of OEHHA 2015) was conducted in steelhead trout, which is of limited relevance to humans. Some of the study results were not consistent with that in the literature. For example, yolk sac edema, which had been observed in similar PCP studies in the past, was largely absent in this study. The lower concentrations of PCP actually stimulated the growth.

**Hodson et al. (1981)** (page 45 of OEHHA 2015) was conducted in rainbow trout, which is of limited relevance to humans. The objective of this study was to investigate the interaction between temperature and PCP toxicity to early life stages of rainbow trout.

**Wu et al. (2009)** (page 34-35 of OEHHA 2015) is an article published in a Chinese journal. The study used a cDNA microarray technology. The biological relevance is questionable and extrapolation to humans is unclear. PCP was used to treat the fish embryos but only one concentration (50 µg/L) was

used and only one time point (8 hours) was observed. No rationales for selection of the concentration and time point were provided. As such, the reliability of the study result would be discounted. The biological relevance of changes in gene expression of such a great number of genes is questionable. (A total of 1149 transcripts were significantly up-regulated while 501 transcripts were down-regulated).

(2) **In vitro studies or assays in mammals** are of potential use for mechanistic or screening purposes. As such they might be sentinel studies with greater sensitivity than mammalian studies and thus may identify the need to conduct mammalian studies. While this information may offer valuable insights on the mechanism of action of PCP in this area of reproductive toxicity, it plays little if any role in aiding the evaluation of reproductive effects in humans. These studies might offer some limited support according to 3 B. of the "reproductive criteria" but **only limited support.** The following studies cited in OEHHA 2015 fall into this category.

**Group II- In vitro mammalian studies**

**Lin et al. (2009)** Rhesus Macaque sperm (page 31 of OEHHA 2015)

**Osterloh et al. (1982)** Mouse sperm (page 32 of OEHHA 2015)

Both these studies were one of a kind, and do not add any information of potential use in human assessment.

The Lin et al. study was a mechanistic study using an MRI technique to analysis the composition of metabolites resulting from the exposure of macaque sperm to PCP and other chemicals. Notably, the PCP samples could not be distinguished from the controls.

The Osterloth et al. study was an attempt to repurpose a study designed to detect mutagens to also detect testicular toxicity. Several pesticides were tested and none produced significant results relative to vehicle controls.

**Yang et al. (2005)** (page 19 of OEHHA 2015) is an article published in a Chinese journal. The study used the sertoli cells directly isolated from rats and measured the cell viability by PCP treatment ex vivo. The MTT assay used for measuring the cell viability is a general assay for assessing cell metabolic activity and measuring cytotoxicity. The assay is not specific for the reproductive cells. In other words, if PCP would be treated to other cell types (i.e., fibroblasts), it is likely that similar results would be obtained. As such, this is an ex vivo study using rat sertoli cells that is of limited contribution for the evaluation of humans. The MTT assay used is not targeted or specific to the reproductive or developmental endpoint and it only showed at most that PCP under the test condition affected cell viability.

**Gravance et al. (2003)** (page 19-20 of OEHHA 2015) is a proof of concept study regarding methods development. PCP is used as a reference control using a limited dose curve (0.1 μM, 1.0 μM); no information is provided regarding the relationship of dose to actual human exposure. No details are provided on purity of the PCP or on the rats from which the spermatozoa were sourced. The reported methods and endpoints examined are limited (mechanistic information regarding uncoupling of oxidative phosphorylation).

**Seibert et al. (1989)** (page 25 of OEHHA 2015) provides information on method development (evaluation of sperm swimming activity and sperm motion in vitro as a sensitive and potentially valuable endpoint) and uses high in vitro doses, bovine spermatozoa, without comment on the relationship to human or environmental exposure. The study represents mechanistic information which is difficult to relate based on in vitro dosing. Additionally, findings in the multigenerational rat study by Bernard, et al. suggest doses of PCP orally (≥30 mg/kg bw) affect male reproductive indices (spermatid counts, weights of prostate and testes, fertility), but data on P1 and F1 male rats did not indicate abnormal sperm or changes to percent motility. This may be due to differences in measurement, sensitivity of the animal model; however, the in vitro application must also be considered.

**Fiorini et al. (2004)** (page 38 of OEHHA 2015) is designed to evaluate the utility of an in vitro model regarding molecular markers for toxicity in sertoli cells (SerW3). The authors looked at a number of compounds, including PCP (purity not stated). Results of PCP (0.1 uM) on protein expression (Western blot analysis of occludin, ZO-1, N-cadherin and Cx43 were negative; additionally, PCP had no marked delocalization effect on immunoreactivities of ZO-1 and n-cadherin. This study provides information regarding mechanistic targets in sertoli cells, for which, in this study, PCP appears to be without remarkable findings.

**Owens et al. (2000)** (page 40 of OEHHA 2015). The relevance of the animal model (Japanese medaka) to human risk assessment is unclear. An additional limitation of the study is the lack of purity analysis of the test substance, the relevance of the dose range to human in vivo exposure with regard to extrapolation of the direct in vitro exposure (120 ng/egg and higher). Additionally, it is difficult to weigh the findings due to the fact that no effects were observed in non-lethal concentrations.

**Helmstetter et al. (1995)** (pages 40-41 of OEHHA 2015). While this study appears to be a well done environmental methods development study, the animal model used in this study cannot be easily extrapolated to human risk assessment.

**Group III - In vivo assays**

**Hinkle (1973)** (page 46 of OEHHA 2015) conducted a one generation reproductive study in hamsters with PCP. The dose range used was 1.25 to 20 mg/kg bw/day and some deaths and fetal resorptions were observed at doses not given. The study did not many of the features of modern studies and we don't know the number of animals and other information. No NOAEL was given in the abstract.

**Chou et al. (1979)** (page 45 of OEHHA 2015) conducted a reproduction study in rats with pure and technical grade PCP at doses of 0, 0.4,4 or 40 mg/kg/day given intraperitoneally. The relevance of the route of exposure to humans is unclear.

**Beard et al. (1999)** (page 22-23 of OEHHA 2015). Weakness of the study include the lack of information regarding a dose response curve (one dose study), purity of the PCP and use of few animals (the control group consisted of 6 and the PCP treated was 13).

**Exon et al. (1982)** (page 26 of OEHHA 2015). Limitations of the study include the lack of clear methodology and documentation. Animals selected for the study were weaned from dams at day 21, but there is no description of how litters were divided to avoid cofounding effects of littermates. There

appears to be some disparity regarding the number of replicates with the methods indicating groups of 12 – 20 rats, but the tables indicate numbers of examined as 12 – 14/group. Animals were bred at 90 days of age, but no details are provided on the sourced males. Statistical methods are not described. Several of the observations noted by the authors appeared to lack a dose response (e.g., the authors note that percent of stillborn pups born to dams treated with PCP was generally greater than controls; though true, the values were 1, 7, 2, 3%, for 0, 5, 50 and 500 ppm PCP, respectively). Feed intake was not measured and intake (mg/kg bw) was not calculated; furthermore, accurate estimates of compound intake are complicated by the group housing (4/cage) employed. Additionally, the authors note that purity of the test compound was not as supplied (stated as 95%, but measured as 85.5%) and content of dioxins ~ 400 ppm. Levels of PCP were measurable in basal feed, as well as liver and kidney from control animals. It appears that the background level in kidney was similar to animals consuming PCP feed and the levels in liver do not indicate a dose response with increased feed concentration. The reported findings occurred in the absence of maternal toxicity (evaluated as a lack of effects on body weight gain of dams); this contradicts other studies which demonstrate a lack of findings at non-maternal doses. Due to some of the reporting limitations, this study is limited to supporting data.

**Larsen et al. (1975)** (page 26 of OEHHA 2015). Weaknesses of the study include the fact that only one dose was utilized, (rationalized as 3/4th of the LD50), additional tissues were not collected beyond the maternal/fetal compartment and serum, a mass balance was not conducted, and the number of replicates was low (2/time-point). The authors conclude that the amount transferred to the fetus was considered negligible, with a peak in the placenta and fetuses at 12 hours as compared to 8 hours in the maternal blood. The teratology study, while interesting and using a high purity sample, used a low number of replicates limiting its power. The authors combined the findings in the two studies to suggest indirect teratogenic activity through maternal toxicity based on the small amount of placental transfer. The collective studies can be considered pilot in nature and supporting.

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In sum, of the forty three studies and abstracts cited in the OEHHA 2015 document, thirty four can be discounted for the reasons given above.

If you have questions, please do not hesitate to contact the undersigned.

Respectfully submitted,

Roger C. Jackson