August 16, 1989

Tox. Chem. No. 641

MEMORANDUM

SUBJECT: TB Project Nos. 8-0418, 19, and 20.
Pentachlorophenol: Reviews of Open Literature Articles on Developmental Effects (1) and Mutagenicity (2).

TO: Spencer L. Duffy, Review Manager
Special Review Branch
Special Review and Reregistration Division (H7508C)

FROM: David G. Van Ormer, Ph.D. DVO 08-16-89
Science Administration Section
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THRU: Robert Coberly RC 8/17/89
Head, Science Administration Section
Science Analysis and Coordination Branch, HED (H7509C)

The attached DER's pertain to journal articles which were noted in our Memo for TB Proj: No. 7-0988 as worthy of our review. The studies do not indicate any particular further concern regarding the hazards of Penta. The articles do, however, reinforce our knowledge of the toxicity of Penta in the categories of developmental effects and mutagenicity.

We are retaining the articles for our files. Many thanks for your assistance in obtaining copies of these studies for our use.

Reviewed By: David G. Van Ormer, Ph.D. DVO 04-14-89
Science Administration Section, SACB (H7509C)
Secondary Reviewer: Robert Coberly
Science Administration Section, SACB (H7509C)

007433

DATA EVALUATION REPORT
(Summary of Journal Article)

Study Type: Teratogenicity With Subchronic Feeding

TOX Chem No.: 641 and 638F
MRID No.: N/A

Test Material: Pentachlorophenol; Pentachloroanisole (both purified)

Synonyms: PCP, Penta; PCA

Testing Facility: Food and Drug Administration, Washington, DC

Title of Article: Teratogenic Potential of Purified Pentachlorophenol and Pentachloroanisole in Subchronically Exposed Sprague-Dawley Rats.

Authors: J.J. Welsh, T.F.X. Collins, et al.

Journal: Food Chemistry and Toxicology 25(2), 163-172 (1987)

Conclusions:

1. Pentachlorophenol

   NOEL, maternal toxicity = 4 mg/kg/day (LDT).

   LEL, maternal toxicity = 13 mg/kg/day (increased resorptions).

   Maternal effects at top dose (43 mg/kg/day): reduced body weight (adjusted).

   NOEL, developmental toxicity: reserved pending availability of individual fetal data.

   Developmental effects at mid-dose (13 mg/kg/day): reduced fetal body weight, reduced crown-rump length, increased incidence of misshapen centra, increased overall skeletal variations.

   Developmental effects at top dose (43 mg/kg/day): markedly reduced number of viable fetuses.

2. Pentachloroanisole

NOEL, maternal toxicity = 12 mg/kg/day (mid dose).

LEL, maternal toxicity = 41 mg/kg/day (HDT): increased resorptions, reduced body weight gain (adjusted), fewer corpora lutea.

NOEL, developmental toxicity = less than 4 mg/kg/day (LDT).

LEL, developmental toxicity = 4 mg/kg/day: decreased fetal weight and crown-rump length in males (also at top dose).

Other effects at top dose (44 mg/kg/day): markedly reduced number of viable fetuses, increased sternebral and other skeletal variations.

Before recording a developmental NOEL from this study for penta, HED requests availability of the individual data for fetal body weights. The data of this study, however, do not appear to give concern regarding the current penta NOEL (developmental toxicity) at 3 mg/kg/day.

The 6-month treatment period is much longer than for a standard teratogenicity study. The classification of Supplementary reflects the ancillary value of the present study as either a teratogenicity or subchronic feeding study.

Classification: Supplementary

Purified pentachlorophenol (penta) and purified pentachloroanisole (PCA; a penta metabolite in some biological systems) were separately administered to male and female Sprague-Dawley rats for 181 days, through mating and pregnancy. The analysis of penta showed octachlorodibenzodioxin (1.25 ppb) as the only detected impurity. The daily dietary intakes of penta were 0, 4, 13, or 43 mg/kg, and of PCA were 0, 4, 12, or 41 mg/kg. Each dose group contained 20 rats of each sex (32 to 34 days old), except the control group, which contained 40 rats of each sex.

Neither of the chemicals produced adult mortality. Signs present in the high-dose penta dams: 50 percent showed "ringed eye" and 25 percent showed vaginal bleeding.

Food consumption was generally elevated (relative to controls) in all penta treatment groups throughout pregnancy. Food consumption showed little change in the PCA groups.

At the end of pregnancy, the female body-weight gain (unadjusted) was slightly elevated at penta low and mid doses, but markedly reduced at top dose. When adjusted for gravid uterus weight, the patterns of body weight gains for either the penta or PCA dams were similar to the pattern of unadjusted weight gains for penta. Pregnancy rate (as affected by either compound) was slightly reduced at low dose and slightly elevated at the top two doses.

The number of corpora lutea was significantly reduced at top dose of the PCA animals only. The number of viable fetuses was significantly and markedly reduced at top dose for either compound, and also somewhat reduced at the PCA mid dose. Either compound at top dose showed marked embryolethality, with the lower dose levels unaffected. For penta at top dose, 16/17 litters were totally resorbed (15/18 for PCA). The penta mid dose showed 81.25 percent of litters with two or more resorptions (control, 41.94 percent).

Penta produced a dose-related decrease in fetal body weight, significant at mid dose and also reduced at low dose in either sex.

Dose Penta (mg/kg)	Mean Body Weight (g)	
	Male	Female
0	4.25	3.98
4 (60 ppm)	4.06	3.87
13 (200 ppm)	3.81*	3.65*
43 (600 ppm)	2.70**	----

*$p < 0.05$
**Not analyzed statistically due to small sample size.

Crown-rump length was reduced at mid dose in either sex of the penta animals. When treated with PCA, either low-dose or top-dose male fetuses exhibited significant reductions in fetal weight and crown-rump length.

For either compound, the dietary exposure was unrelated to specific external or sternebral variations. At mid dose, the fetuses exposed to penta showed an increase in misshapen centra, the only specific skeletal variation significantly increased over controls. With regard to overall skeletal variations among penta fetuses, there were significant increases at mid dose for mean fetuses per litter having at least one or (separately) two skeletal variations, and for percent litters with fetuses having at least two skeletal variations.

There were neither specific nor overall changes in soft tissue variations that could be related to either penta or PCA treatment.

The extreme embryolethality at top dose prevented inclusion of results at that level in the statistical analysis (except for implantation, survival, and resorption data).

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DATA EVALUATION REPORT
(Summary of Journal Article)

Study Type: Mutagenicity - HGPRT Locus

TOX Chem No.: 641
MRID No.: N/A

Test Materials: Pentachlorophenol and (separately) Three Associated Chlorophenols

Testing Facility: Departments of Biology and Chemistry, University of Jyvaskyla

Title of Article: Mutagenesis of Mammalian Cells in Culture by Chlorophenols, Chlorocatechols and Chloroguaiacols.

Authors: M.L. Hattula and Juha Knuutinen

Journal: Chemosphere 14(10), 1617-1625 (1985)

Conclusions:

Eleven chlorophenolic compounds and one related wood preservative mixture were tested for forward mutation at the HGPRT locus in V79 cells. Assay was performed both with activation (rat fibroblasts or primary rat hepatocytes) and without activation (V79 cells alone).

Of the chlorophenols known (to the reviewer) to be contaminants of penta, the 2,3,4,6-terachlorophenol was the only active compound in any of the three assay methods. Mutants were produced in the unactivated (but not the activated) V79 cells, at a maximum frequency which was less than 1.0 percent (on a weight basis) of the frequency of the positive control (MNNG). The dose response spanned three doses: 3.5 to 10 ug/mg at a cloning efficiency of 71 to 75 percent. At the LDT (3.5 ug/mg) there were 12 mutants per 10^6 survivors, compared with zero in control and 799 for the positive control at 1.0 ug/mg.

Pentachlorophenol was inactive by all three methods.

Classification: Supplementary

Abstract: Several chlorinated phenolic compounds were tested for mutagenicity (forward mutation). Each of four chlorinated phenols, four chlorinated catechols, three chlorinated

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guaiacols, one chloromethoxyphenol, and a wood preservative mixture (KY-5, containing mainly 2,4,6-TCP, 2,3,4,6-tetra CP, and some methoxyphenols) were assayed separately for production of thioguanine resistance (forward mutation at the HGPRT locus) in the Chinese hamster cell line, V79.

Note: HGPRT is a non-essential enzyme in the wild type which can poison the cell by producing thiohypoxanthine from 6-thioguanine. The mutant, not endowed with HGPRT, survives when cultured with 6-thioguanine (and is designated $6TG^R$).

Three separate induction conditions were attempted as follows:

1. Direct method without metabolizing cells.
2. Co-cultivation with rat fibroblasts.
3. Co-seeding with rat hepatocytes.

In the "direct method" the test compound was added to the tissue culture dish 24 hours after the V79 cells (10^6 cells in 10 mL medium). Controls received the corresponding total volume of acetone (solvent control). After incubation, the cells were dissociated (trypsin-EDTA) and seeded at appropriate concentrations for determining the cloning efficiency. Culture dishes each received a seeding of 2×10^4 cells (in 4 mL of medium) to which 6-thioguanine was added at a final concentration of 40 uM. Colony counting followed Giemsa staining. The colony count in five dishes per point (7 to 8 days after cell seeding) gave a value for the cloning efficiency. The counts from 16 dishes per point, 12 to 14 days after cell seeding, yielded the frequency of 6-thioguanine-resistant mutants. For the direct method the positive control was MNNG.

In the cell-mediated (co-cultivation) method, the test chemicals were separately co-cultivated with irradiated rat fibroblasts (monolayer of 2×10^6 cells) on which were seeded 3×10^5 V79 cells. The positive control was DMBA, and the experiment was conducted as in the direct method.

For the hepatocyte-mediated (co-seeding) assay the primary hepatocytes from 2- to 3-month-old male rats (Sprague-Dawley) were employed, and only one concentration of each of the eight test compounds was tested. Positive control was DMN. The V79 cells were seeded at 5×10^5 cells/25 cm^2 T-flask. After 18 hours, the primary rat hepatocytes (2×10^6) were seeded onto the V79 cells in 4 mL of Leibovitz L-15 medium (containing glutamine, fetal calf serum, penicillin, and streptomycin). After a change of medium, the respective chlorophenols were added, and the cells (after 18 hours) were dispersed with trypsin and EDTA. The experiment was conducted as in the direct method.

Results and Reviewer's Discussion:

The only specific chlorophenol congeners which are known to the Reviewer* to be concomitant to penta are 2,4-dichlorophenol and the two tetrachlorophenols: 2,3,5,6- and 2,3,4,6-. In the present journal article, the only one of these three compounds which showed activity was 2,3,4,6-tetrachlorophenol, and then only in the direct method (without metabolizing cells). The strength of its mutagenic activity was over one hundredfold weaker (on a weight basis) than the positive control, MNNG.

Penta, itself, was negative by all three assay methods.

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*"Chlorophenols and Their Impurities in the Canadian Environment: 1983 Supplement," Environmental Protection Programs Directorate (March 1984), page 4.

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DATA EVALUATION REPORT
(Summary of Journal Article)

Study Type: Mutagenicity - Survey of Literature

TOX Chem No.: 641
MRID No.: N/A

Test Material: Pentachlorophenol, Technical

Synonyms: Penta, PCP

Title of Article: "A Methodology for Assessing Mutagenic Hazards of Chemicals."

Authors: H.S. Brown, D.R. Bishop, and C.R. West

Journal: Toxicology and Industrial Health 2(3), 163-182 (1986)

Conclusions:

From a survey of the published literature, pentachlorophenol was categorized in mutagenicity hazard category D (limited evidence), based on the mutagenicity test results as follows:

Positive

1) S. cerevisiae, homozygosis - gene conversion.

2) S. cerevisiae - forward mutation.
3) Cell transformation - Syrian hamster embryo (enhancement of viral transformation).

Negative

1) Mouse spot test.

2) Ames test (S. typhimurium, histidine reversion).

3) Host mediated assay.

All the above studies are cited in the Wood Preservatives PD 1, except the cell transformation study, which is cited in Casto, Bruce C. (1976) Progress Report NIH-NCI-NO1-CP-45615, pages 1-18. Bioresearch International, Powell, OH 43065.

The test system (SA7/SHE) for the transformation study measured the enhancement of simian adenovirus (SA7) induction of transformation in Syrian hamster embryo cells, which had received prior exposure to a putative chemical carcinogen. The dose range was 6 to 100 ug penta/mL. The result was positive, with the lowest transforming concentration at 50 ug penta/mL.

Classification: Supplementary

Description of the Survey:

Fifty-one chemicals were classified into "mutagenic hazard categories," based on the results (positive or negative) of the mutagenicity data used. The principal sources were the EPA GENE-TOX database and the IARC Monographs. For the group of chemicals as a whole, seventy-five different assays were included. The assays were grouped according to test-significance, on the criteria of sensitivity, specificity, validity, reliability, and relevance to humans. Each chemical was assigned to a hazard category, labeled A (most hazardous) through E, after considering variables as follows: the number and type of endpoints measured, the number and type of species represented, the significance of postive and non-positive results, the relevance of specific tests for predicting effects in humans, the test-significance level of each test result, and the overall pattern presented for the chemical.