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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 12 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 063001. Pentachlorophenol. Review of In Vivo Mouse Micronucleus Test for Reregistration

PC Code 063001
Tox. Chem. No. 641
Project No. D223149
Submission No. S500556

Case No. 807487
Reregistration Case No. 2505
ID No. 063001-005382
MRID No. 43911301

TO: Mark Wilhite, CRM Team # 53
Special Review and
Reregistration Division (7508W)

Jane Smith
Reregistration Section
Risk Characterization and
Analysis Branch
Health Effects Division (7509C)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)

Pamela M. Hurley 3/19/96

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)

Roger Gardner 3/20/96 RLG 3/18/96

Background and Request:

The Pentachlorophenol Task Force/SRA International has submitted an in vivo mouse micronucleus test in bone marrow cells in support of reregistration. The Toxicology Branch (TB-I) has been asked to review the study and determine whether or not it is acceptable for reregistration purposes.

Toxicology Branch Response:

The Toxicology Branch has reviewed the submitted study and has determined that it is acceptable for regulatory purposes. The study satisfies the guideline requirement 84-2 for in vivo cytogenetic mutagenicity data. The following paragraphs summarize the results of the study.

(1)

In a CD-1 mouse bone marrow micronucleus assay (MRID 43911301), 5/sex/dose were treated by gavage with pentachlorophenol (88.9% a.i.) at doses of 24, 60, or 120 mg/kg for males and 20, 50 or 100 mg/kg for females. Bone marrow cells were harvested at 24, 48 and 72 hours post-treatment. The vehicle was corn oil. The positive control was triethylenemelamine (TEM).

There were clinical signs of toxicity during the range-finding study. These included prostration, ataxia, salivation, convulsions and death. Death was also observed in the high dose males of the main study. Penta was tested at an adequate dose based on clinical signs of toxicity and death at higher doses. The positive control induced the appropriate response. **No significant increases in the frequency of micronucleated polychromatic erythrocytes were observed in the bone marrow at any of the harvest times.**

The following list of studies and footnotes summarize the current status of the toxicology studies for penta.

		Required[1]	Satisfied
81-1	Acute Oral Toxicity	Yes	Tentatively[2]
81-2	Acute Dermal Toxicity	Yes	Tentatively[2]
81-3	Acute Inhalation Toxicity	Yes	Waived[3]
81-4	Primary Eye Irritation	Yes	Tentatively[2]
81-5	Primary Dermal Irritation	Yes	Tentatively[2]
81-6	Dermal Sensitization	Yes	Yes
81-7	Acute Delayed Neurotoxicity	No	-
82-1(a)	Subchronic Oral (rodent)	Yes	Pending[4]
82-1(b)	Subchronic Oral (non-rodent)	Yes	Pending[5]
82-3	90-Day Dermal	Yes	Yes
82-4	90-Day Inhalation	Yes	Waived[3]
83-1(a)	Chronic Toxicity (rodent)	Yes	Pending[4]
83-1(b)	Chronic Toxicity (nonrodent)	Yes	Pending[5]
83-2	Oncogenicity (mouse)	Yes	Yes[6]
83-5	Oncogenicity (rat)	Yes	Pending[4]
83-3(a)	Teratology (first species)	Yes	Yes
83-3(b)	Teratology (second species)	Yes	Yes
83-4	Multigeneration Reproduction	No	-[7]
84-2(a)	Mutagenicity - Gene Mutation	Yes	Tentatively[8]
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	Yes
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	Tentatively[8]
85-1	Metabolism	Yes	Tentatively[9]
85-2	Dermal Penetration	Yes	Tentatively[10]

Comments:

1. These requirements were taken from the FIFRA '88 Data Call-In (taken from 48-hour review).

2. Acute oral: The acute oral studies are all literature studies. One study was conducted with mice. All the studies appear to support Toxicity Category II, although none of the studies are guideline studies.

   Acute dermal: There is a study that had 4 males/group with a six hour/day exposure period for 4 days. The dose levels administered were up to 1300 mg/kg, but these animals had already been exposed to PCP several times in corn oil and methylcellulose prior to this time in the same study. This study supports at least Toxicity Category II. In a second acute dermal study, 4 rabbits were tested, sex unknown. One of 4 rabbits died at 2000 and 3980 mg/kg, each. The LD_{50} was greater than 3980 mg/kg. This study supports Toxicity Category III. The problem with these studies is that only one sex is known.

   Primary eye irritation: The eye irritation study only observed the animals up to 7 days. The current guidelines state that the animals should be observed up to 21 days. The Toxicity Category could be overexaggerated since there were still effects at 7 days.

   Primary skin irritation study: The submitted study is a 21-day dermal study. TB-I will have to look at this one more closely in order to determine a possible toxicity category.

3. Since the 90-day inhalation study has been waived by the Greybeard Committee for inability to generate respirable vapors or dust, TB-I is also waiving the acute inhalation study for the same reasons.

4. The chronic feeding study in the rodent is currently being conducted at The National Toxicology Program (NTP). If this study is acceptable, then the subchronic study will not be required. Also, if the NTP has the chronic study, it probably has the subchronic study as well, and that may be acceptable for the subchronic requirement. In addition, there are some subchronic feeding studies that were submitted for the reproduction requirement that might be acceptable.

5. This study will not be required if the chronic dog study is acceptable. The chronic dog study is in progress.

6. An NTP study has been conducted on the mouse and the Agency had accepted it. It appears that a DER was not written for this study and that the Office of Pesticide Programs (OPP) used something that was written by another part of the Agency.

7. The requirement for a 2-generation reproduction study has been waived. It has been determined that for the purposes of risk assessment, the results from the one-generation reproduction studies may be used with an additional uncertainty factor of 3 because of a concern over the possibility of observed reproductive effects at lower dose levels in the second generation.

8. There are NTP studies available for gene mutation, sister chromatid exchange and chromosomal aberration. Based on a preliminary assessment, the summaries for these mutagenicity studies are tentatively acceptable. These studies would satisfy all the requirements for the old mutagenicity testing guidelines. An acceptable in vivo mouse micronucleus study is available. This study satisfies the mutagenicity requirement for an in vivo chromosomal aberration study under the new mutagenicity testing guidelines. It also satisfies the requirements for a chromosomal aberration study under the old guidelines. At one time, the Task Force also submitted a protocol for an in vitro mouse lymphoma study. If the Task Force completes this study, it will have completed all the mutagenicity testing requirements under the new mutagenicity testing guidelines.

9. The metabolism studies are literature studies. They are tentatively acceptable.

10. The dermal absorption study is also a literature article. This study was quickly reviewed by the TB-I expert on dermal absorption who stated that it is a limited study but is tentatively acceptable.

[PENTACHLOROPHENOL] MICRONUCLEUS (84-2)

EPA Toxicologist: Pamela M. Hurley Pamela M Hurley, Date 3/19/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 3/20/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice; OPPTS 870.5395 [§84-2]

DP BARCODE: D223149 SUBMISSION CODE: S500556
P.C. CODE: 063001 TOX. CHEM. NO.: 641

TEST MATERIAL (PURITY): Pentachlorophenol (88.9%)

SYNONYMS: Penta

CITATION: Xu, J. (1996) In vivo test for chemical induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells. SITEK Research Laboratories, Rockville, MD. Study Number 0371-1521, January 20, 1996. MRID 43911301. Unpublished.

SPONSOR: The Pentachlorophenol Task Force, c/o SRA International, Inc. 1850 M Street, N.W. Washington, D.C. 20036

EXECUTIVE SUMMARY:

In a CD-1 mouse bone marrow micronucleus assay (MRID 43911301), 5/sex/dose were treated by gavage with pentachlorophenol (88.9% a.i.) at doses of 24, 60, or 120 mg/kg for males and 20, 50 or 100 mg/kg for females. Bone marrow cells were harvested at 24, 48 and 72 hours post-treatment. The vehicle was corn oil. The positive control was triethylenemelamine (TEM).

There were clinical signs of toxicity during the range-finding study. These included prostration, ataxia, salivation, convulsions and death. Death was also observed in the high dose males of the main study. Penta was tested at an adequate dose based on clinical signs of toxicity and death at higher doses. The positive control induced the appropriate response. **No significant increases in the frequency of micronucleated polychromatic erythrocytes were observed in the bone marrow at any of the harvest times.**

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: pentachlorophenol
Description: small, light tan pellets
Lot/Batch #: EL-064
Purity: 88.9% a.i.
Stability of compound: assumed stable at room temperature because instructions from Sponsor stated to store at room temperature; keep out of direct sunlight
CAS #: 87-86-5
Solvent used: corn oil
Other comments: none

2. Control Materials:

Negative/Route of administration: N/A

Vehicle/Final volume/Route of administration: corn oil; 10 mL/kg by oral gavage

Positive/Final dose(s)/Route of administration: triethylenemelamine (TEM); 1.0 mg/kg, 10 mL/kg by intraperitoneal injection

3. Test compound administration:

Volume of test substance administered: 10 mL/kg in corn oil

Route of administration: oral gavage

Dose levels used: range-finding study: 10, 50, 100, 500, 1000 and 2000 mg/kg (solubility limit). Main study: 24, 60, or 120 mg/kg for males and 20, 50 or 100 mg/kg for females based on toxicity.

4. Test animals:

a. Species mice Strain CD-1 Age 42 days
Weight male 25-34 grams female 18-24 grams
Source: Charles River Laboratories, Raleigh, North Carolina

b. No. animals used per dose at each harvest time: 5 males 5 females

c. Properly maintained? Yes

6

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Test compound
Dosing: x once _ twice (24 hr apart) _ other

Sampling (after last dose): _____ 6 hr _____ 12 hr ____x 24 hr ____x 48 hr ____x 72 hr (mark all that are appropriate), _____ other (describe):

b. Negative and/or vehicle control
Dosing: ____x once _____ twice (24 hr apart) _____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr ____x 24 hr ____x 48 hr ____x 72 hr (mark all that are appropriate), other (describe):

c. Positive control
Dosing: ____x once _____ twice (24 hr apart) _____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr ____x 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate), other (describe):

2. Tissues and Cells Examined:

____x bone marrow _____ other (list):

Total number of polychromatic erythrocytes (PCE) in approximately 1000 erythrocytes per animal were examined.
Total number of normochromatic erythrocytes (NCE; more mature RBCs) in approximately 1000 erythrocytes were examined per animal.
Also, the number of micronucleated PCE (MCPE) and NCE per 2000 PCE was determined.

3. Details of slide preparation: The mice were sacrificed by CO_2 asphyxiation. The bone marrow from the femurs of each mouse was removed by flushing into a 15 mL centrifuge tube using a 1-cc syringe containing a small volume of fetal bovine serum. The tubes were centrifuged at 800 rpm, the supernatant was removed, leaving approximately 0.1 mL above the cell pellet and the cells were resuspended by flicking the tube into a suspension. A small drop of the suspension was placed on a microscope slide and spread along the length of

the slide. The slides were allowed to air dry, fixed in methanol for 15 minutes and then allowed to air dry again. The slides were then stained in Wright-Giemsa stain for 3-4 minutes, rinsed in distilled water, allowed to air dry completely and then mounted in Permount using #1 cover glasses. The slides were scored "blind" in order to avoid bias.

4. Statistical methods: The data for males and females were calculated separately. The report stated that "unless otherwise indicated, the frequency of MCPE in each dose group was compared to that in the respective vehicle control group using a two-tailed Student's t-test. Results were considered significant if the p value was ≤ 0.05. Statistical analysis was not performed on values that were lower than or equal to those of the respective vehicle control."

5. Evaluation Criteria

The report listed criteria for a valid assay, positive response, negative response and equivocal response. These criteria are as follows:

1. "In the vehicle control, the average number of MPCE per 1000 PCE should not exceed 5.

2. In the positive control, the increase in the average number of MPCE per 1000 PCE over the average number of MOCE for the vehicle control should be statistically significant.

3. At least three animals from each sex must be alive at the time of sacrifice for each dose level.

The test article is considered to have caused a positive response in this assay if:

1. The test article shows a positive dose-response trend and a statistically significant increase in the number of MPCE at one or more dose levels over that of the concurrent vehicle control. In the event that the test article causes a statistically significant increase in the number of MPCE due to an unusually low number of MPCE (less than 0.05%) in the concurrent vehicle control, the data from that dose may be compared to historical vehicle control data.

8

2. In the event there is no positive dose-response trend, at least two consecutive test doses produce a statistically significant increase in the number of MPCE.

The test article is considered to have caused a negative response if none of the test doses show a statistically significant increase in the number of MPCE when compared to the vehicle control.

The test article is considered to have caused an equivocal response if the test article induces a statistically significant increase in the number of MPCE when compared to the vehicle control at one of the test doses without a positive dose-response trend."

II. REPORTED RESULTS

A. Preliminary toxicity assay: In the range-finding study, the animals were tested at 10, 50, 100, 500, 1000 or 2000 mg/kg. Three animals/sex/dose level were tested. They were observed for 3 days, sacrificed and then slides were prepared. All males and females died at doses of 500, 1000 and 2000 mg/kg and one female died at the 100 mg/kg dose level. All the animals died on day 0, except for 3 males at the 500 mg/kg dose level who died on day 1. In most cases, animal death was preceded by prostration, ataxia, salivation and convulsions. Of the surviving animals, the % mean bodyweight change by 72 hours ranged from 15.4-17.9% in the treated males versus 18.5% in the controls and 20.0% in both the treated and control females. There did not appear to be an effect on bodyweight gain. The PCE/NCE ratios did not show any toxicity due to the test article at any dose level in the surviving animals. The mean ratios were 2.49 (males) and 1.51 (females) in the vehicle control groups and 1.46, 2.12 and 2.44 (males) and 1.76, 1.03 and 1.40 (females) in the 10, 50 and 100 mg/kg groups, respectively.

B. Micronucleus assay: Two males from the 24-hour harvest and 1 male from the 72-hour harvest at the 120 mg/kg dose level died approximately 8 hours after dosing and were replaced with extra animals which had been dosed on the same day and at the same dose level. All animals gained weight. At 24 hours, it appeared that the high dose groups gained less than the respective controls (6.7% versus 10.7% and 9.5% versus 14.3% in males and females, respectively); however, in actuality, the differences in these gains were only about 1-2 grams out of 23-32 grams. The differences in body weight gain were not consistent at 48 or 72 hours. There was no evidence of any significant increase in the number of

MPCE in the treated groups at any dose level and harvest time when compared to the concurrent controls. The positive control, TEM depressed the PCE/NCE ratio and showed a statistically significant increase in the MPCE. The following table summarizes the results.

Mean Ratio of PCE/NCE and Incidence of MPCEs in Bone Marrow of Male and Female Mice Orally Administered Pentachlorophenol

Time (hours)	Dose (mg/kg)	Cell Counts		PCE/NCE ratio	MPCE per 2000 PCEs
		PCE	NCE		
Females					
24	Vehicle*	630	370	1.78	1.2
24	20	579	421	1.40	1.6
24	50	634	366	1.90	1.2
24	100	671	329	2.14	0.6
24	TEM**	302	698	0.45	111.0
48	Vehicle*	622	378	1.86	1.0
48	20	615	385	1.64	1.4
48	50	706	294	2.55	1.4
48	100	627	373	1.92	0.4
72	Vehicle*	743	257	2.91	1.8
72	20	701	299	2.43	0.8
72	50	689	311	2.26	1.8
72	100	684	316	2.19	1.0
Males					
24	Vehicle*	583	417	1.41	1.8
24	24	599	401	1.60	0.2
24	60	628	372	1.86	1.2
24	120	642	358	2.06	0.8
24	TEM**	201	799	0.26	78.4
48	Vehicle*	565	435	1.43	1.4
48	24	597	403	1.58	1.8

Mean Ratio of PCE/NCE and Incidence of MPCEs in Bone Marrow of Male and Female Mice Orally Administered Pentachlorophenol					
Time (hours)	Dose (mg/kg)	Cell Counts		PCE/NCE ratio	MPCE per 2000 PCEs
		PCE	NCE		
48	60	596	404	1.54	2.2
48	120	517	483	1.12	1.2
72	Vehicle*	634	366	1.77	1.8
72	24	741	259	2.89	0.6
72	60	679	321	2.18	2.6
72	120	682	318	2.19	0.4

*The vehicle is corn oil (10 mL/kg)
**TEM was dosed at 1.0 mg/kg

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

This was a well conducted study. There were no major deficiencies. The test chemical was tested up to a level of toxicity. There was no statistically significant increase in the number of MPCE up to levels of systemic toxicity. The positive control induce an appropriate positive response.