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

MAY 24 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id# 109701. Permethrin. Review of a mouse micronucleus assay in vivo.

TOX CHEM No.: 652BB
PC No.: 109701
Barcode No.: D190324
Submission No.: S438931

FROM: John Doherty 5/13/93
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Linda DeLuise/Jay Ellenberger
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Registration Division (H7505C)

THROUGH: Marion Copley, DVM, Section Head
Section IV, Toxicology Branch I
Health Effects Division (H7509C)
Marion Copley 5/17/93

I. CONCLUSION

Toxicology Branch I has reviewed the mouse micronucleus study (ZENECA Study No.: CTL/P/3934, March 5, 1993, MRID No.: 427233-02) and has determined that the study is ACCEPTABLE for regulatory purposes. No evidence of chromosomal aberrations was evident in this study. Refer to the DER attached. Additional in vivo mouse micronucleus study data are not required.

II. ACTION REQUESTED

The ICI Corporation has submitted a mouse micronucleus (series 84-2, chromosomal aberration assay) in response to the Agency's request to provide additional mutagenicity/genetic toxicity studies. The study was reviewed and the copy of the DER is attached.

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[84-2, Mouse micronucleus-Permethrin/1993]

Reviewed by: John Doherty 5/13/93
Section IV, Toxicology Branch I (H7509C)
Secondary reviewer: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: 84-2. Mouse micronucleus test.

MRID NO.: 427233-02 **TOX. CHEM. NO.:** 652BB
PC No.: 109701

TEST MATERIAL: Permethrin technical batch No.: P58/D7534/30.

STUDY NUMBER(S): CTL/P/3934

SPONSOR: ZENECA, Inc. Ag Products, Wilmington, Del.

TESTING FACILITY: Zeneca Central Toxicology Laboratory, Cheshire United Kingdom

TITLE OF REPORT: "Permethrin: Evaluation in the Mouse Micronueleus (sic) Test"

AUTHOR(S): D. Fox and J.M. MacKay

REPORT ISSUED: March 5, 1993

STUDY DATES: September 21 to October 2, 1992 - in-life phase; Slides evaluated November 9 to 20, 1992.

CONCLUSIONS:

No evidence of chromosomal aberrations was demonstrated.

CD-1 strain mouse. Single oral dose levels tested: Males 200 mg/kg, females: 320 mg/kg in corn oil.

Classification: ACCEPTABLE.

Quality Assurance Statement: Provided
Good Laboratory Practice Statement: Provided

REVIEW

Experimental Constants:

Test Chemical: Technical grade permethrin from batch No.: P58/D7534/30, described as an amber liquid and obtained from ICI Agrochemicals, Berkshire, UK. The test sample was assigned a certified purity of 93.1% w/w (an analytical certificate was not provided with the study report).

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Cyclophosphamide of unstated purity was used as the positive control.

Permethrin was administered orally in corn oil (10 ml/kg). The positive control was administered in saline.

Test System: Male and female CD-1 mice (4-7 weeks old) were provided by the Charles River Breeding Laboratories Margate, UK.

Basic Experimental Design:

A. Determination of the Maximum Tolerated Dose.

Groups of 2 male and 2 female mice were dosed with 320 mg/kg. One male died and both females survived. A group of 5 males and 5 females were then dosed with 200 mg/kg; all survived. Two groups of 2 females were dosed with 500 mg/kg and 3 of the 4 died. On this basis it was determined that dose levels of 200 for males and 320 for females mg/kg would be the appropriate dose levels for the main study. No information was presented on the behavioral reactions their time to onset and duration or the time of death due to treatment. Previous experience with permethrin administration in corn oil indicates that the onset of symptoms would be within a few hours after compound administration.

B. Main Study.

Five groups each consisting of five male and five female mice (total of 50 mice) were dosed as either corn oil control, cyclophosphamide (65 mg/kg) and permethrin (200 mg/kg for males and 320 mg/kg for females). There were 2 groups of control and permethrin treated mice and a single group of cyclophosphamide treated mice. The control and permethrin treated mice were sacrificed 24 and 48 hours after treatment, the cyclophosphamide treated mice were sacrificed only after 24 hours of treatment.

The mice were sacrificed at 24 and/or 48 hours after test compound administration. Bone marrow smears (the details of the procedure were provided in the form of an SOP in the Appendix). In summary, the bone marrow preparations were stained with polychrome methylene blue and eosin to visualize the various cell types. One thousand polychromatic erythrocytes per slide were reportedly evaluated for the presence of micronuclei. In addition, 1000 erythrocytes were counted to determine the % of polychromatic erythrocytes in the total erythrocyte population to indicate cytotoxicity in the bone marrow.

ANOVA was used to evaluate the incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample. The values for micronucleated polychromatic erythrocytes were transformed using a square root transformation to stabilize the variance before

analysis. The student's t test (one sided) was also used to assess the data.

Principles of the method. The mouse micronucleus assay is an in vivo method to assess for clastogenic effects of test materials. The test material is administered to the animal and after selected time intervals of 24 and 48 hours, the mice are sacrificed and the bone marrow removed and the blood cells assessed microscopically for indications of chromosomal damage. If chromosomal damage results from a test material, chromosomal fragments lag behind at anaphase. At telophase a large portion of these fragments in not included in the main daughter nuclei which can result in the formation of secondary nuclei or micronuclei. The bone morrow erythrocytes have micronuclei that can easily be detected since the nucleus proper is extruded during maturation of the RBC. Polychromatic erythrocytes are the product of erythroblasts that have expelled their nuclei. The term polychromatic actually refers to the ability of the denucleated cell to react with Romanovsky stains because residues of the expelled nuclei remain with the cell for a short time to react with the stain. Whereas mature erythrocytes appear pink. The polychromatic erythrocytes are considered useful for the detection of clastogenic chemicals because they persist for only 24 hours before maturing into monochromatic erythrocytes. Thus, the micronuclei in these cells will have been produced at the last mitotic division and their formation will be due to the effects of the chemical in the preceding 48 hours.

Results

Tables 1, 2 and 3 photocopied from the study report illustrate the results of this study.

The positive control produced the expected positive result with respect (Tables 1 and 2) to the mean number of micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes (MPE/PE ratio). For example, the males dosed with cyclophosphamide had 15 times more and the females had 28 times more. With respect to the mean % polychromatic erythrocytes (Table 3 an indication of cytotoxicity to the bone marrow), there was an apparent (not reported as being statistically significant) decrease of 31% in males and slight increase (7%) in females.

Both the MPE/PE ratio was approximately doubled for both the males (Table 1) and females (Table 2) dosed with permethrin relative to the control ratio at 24 hours but the difference was not statistically significant. The ratio was less than the control for the 48 hour reading for both sexes. Thus based on this observation, the study author determined that permethrin was not positive in the mouse micronucleus test. Data on the mean percentage of polychromatic erythrocytes in males (Table 3, females were similar) indicated no statistical differences between the animals treated with permethrin and the controls at either time interval but at 24 hours the value was less than the control for both sexes (the high standard deviation of the mean obscured a statistical difference).

CONCLUSION. This study is ACCEPTABLE for regulatory purposes. No evidence of chromosomal aberrations was demonstrated.

Note: This study meets current criteria for acceptability for regulatory purposes. Under the conditions of the study which was conducted at a dose level of about 60% of the estimated LD_{50}, there was no evidence of permethrin induced chromosomal aberration. The study however, is considered a "non-test" for mutagenicity because there is no firm evidence that the test material actually reached the target cells in the bone marrow in effective concentrations to elicit a mutagenic effect. The cytotoxicity test (Table 3) is considered of limited usefulness. For example, the positive control produced the expected positive result with respect to increases in MPE/1000 PE and evidence for chromosomal aberrations but did not cause cytotoxicity in the bone marrow (Table 3). Thus, some chemicals can cause the chromosomal aberrations without causing associated cell toxicity in the bone marrow. The above mentioned problems are inherent in this in vivo assay. The potential for a chemical to cause chromosomal aberrations is better assessed in in vitro experiments.

PERMETHRIN

Page_____ is not included in this copy.

Pages 6 through 8 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Permethrin **BEST AVAILABLE COPY** 010279

MRID # 427233-02

Mouse Micronucleus Test

ZENECA CTL/P/3934

March 5, 1993.

84-2 Mutagenicity Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

General Requirements

1. V Technical form of the active ingredient tested.
2. V Negative, solvent and/or vehicle control(s) for the test system.
3. V Positive control(s) for the test system.
4. V Fully identified test system, species, strain, source etc.
5. V Fully described method for maintaining test system.
6. V Fully described method for preparing test environment and administering test compound.
7. N/A Fully described metabolic activation system, if required.
8. ___ Determination of maximum and range of concentrations/doses used under test conditions.
9. *NO Criteria for determination of a positive effect.

Test Specific Requirements

Salmonella reverse mutation assay

1. ___ Minimum of four strains, TA98, TA100, TA1535 and TA1536. (alternatives need rationale)
2. ___ Strain specific positive controls.
3. ___ Highest concentration limited by toxicity, solubility or 5000 ug/plate.
4. *___ At least 5 different concentrations of test material at adequate intervals.
5. *___ A single positive response confirmed by testing over a narrow range of concentrations.
6. *___ At least three plates experimental point.

Gene mutation in somatic cells in culture

1. ___ Highest concentration limited by toxicity (10-20% relative survival), solubility or 5000 ug/ml.
2. *___ At least 4 different concentrations of test material to yield a concentration related toxic effect.
3. ___ Determination of the number of cell cultures used.

In vitro mammalian cytogenetics

1. ___ Highest concentration limited by toxicity (e.g. reduced mitotic activity; alteration of cell cycle; cytotoxicity), solubility or 5000 ug/ml.
2. [redacted] Multiple concentrations used to define the response.
3. [redacted] At least two independent cultures for each experimental point.
4. [redacted] Determination of culture harvest time.

In vivo mammalian cytogenetics - bone marrow

1. V At least 5 male and 5 female animals per experimental group. (use 48 females giving final n of 4)
2. V Highest dose limited by toxicity or 5000 mg/kg.
3. V Determination of sampling times.

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Aberrations; a) one treatment - 3 times in range of 6-48 hours after treatment adequately spaced with central sample at 24 hour (may be altered based on cell cycle time). b) repeated treatments - samples taken 6 and 24 hours after last treatment (may be

Criteria marked with a * are supplemental and may not be required for every study.

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altered based on cell cycle time).

A → Micronucleus; Samples taken (3) times, starting not earlier than 12 hours after the last treatment and at appropriate intervals following the first sample, but not beyond 72 hours.

4. ✓ Micronucleus assay, at least 1000 polychromatic erythrocytes/animal scored. Ratio of poly to normochromatic determined by counting 200-1000 erythrocytes (1000 OECD).

Rodent dominant lethal assay

1. ___ Sufficient number of dosed males to provide a minimum of 30 pregnant females per mating interval.
2. ___ Concurrent positive control or results from positive control conducted within 12 months in same laboratory with same strain.
3. ___ Highest dose produced toxicity or 5000 mg/kg.
4. ___ Sampling or exposure over entire spermatogenesis cycle of dosed males (8 weeks mice, 10 weeks rats)

Any mutagenicity test with suggestive or greater positive results/activity shall be submitted regardless of missing essential items.

A. Samples of bone marrow taken at 24 and 48 hours only.

Criteria marked with a * are supplemental and may not be required for every study.

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