10/29/91

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

008761

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID # 109701-010182. Permethrin, technical. Review of four mutagenicity studies to support reregistration (resubmission).
Shaughnessey No.: 109701
Tox. Chem. No.: 652BB
Project No.: 1-2252
Submission No.: S384939

TO: Christine Rice, PM Team 52 Product Manager
Linda DeLuise, PM Team 52
Special Review and Reregistration Division (H7508W)

FROM: Linnea J. Hansen, Ph.D.
Toxicology Branch I, Section IV
Health Effects Division (H7509C)
Linnea J Hansen 10/29/91

THRU: Marion P. Copley, D.V.M., D.A.B.T.
Section Head, Toxicology Branch I, Section IV
Health Effects Division (H7509C)
Marion Copley 10/29/91

CONCLUSIONS: The mutagenicity studies submitted to support reregistration of permethrin have been reviewed and are summarized below (DERs attached)

1) Salmonella reverse gene mutation assay: No evidence of increased revertant colonies above control in 5 Salmonella strains up to 5000 ug/plate (solubility limit).
Core-grade: Acceptable MRID 410311-07

2) Unscheduled DNA synthesis, male rat primary hepatocytes: No evidence of unscheduled DNA synthesis above control up to 10^{-4} - 10^{-2} M (limits of cytotoxicity).
Core-grade: Acceptable MRID 409436-04

3) Mammalian in vivo cytogenetics assay, rat bone marrow: No evidence of chromosomal damage up to 6000 mg/kg body weight (single and multiple dose regimes; single sample times).
Core-grade: Unacceptable - no females included in study, only 1 time point sampled in each assay, no preliminary

cytotoxicity data or toxic symptoms at high dose presented. MRID 42142-093

4) Dominant lethal test, mice: No evidence of increased dominant lethal effects up to 150 mg/kg/day (oral dose administered daily for 5 days to males).
Core-grade: Unacceptable - insufficient evidence that high dose was sufficient to test dominant lethal effects. The study can be upgraded if the authors provide data showing systemic toxicity and transport to the target tissues (seminiferous tubules) or evidence that PP557 does not reach the testes in mice because of the blood-testicular barrier. MRID 40943 6-04

The Office of Pesticide Programs is in the process of revising guidelines for mutagenicity studies. The registrant may choose either to resubmit data according to the current guidelines or the revised guidelines, which are not yet in effect.

In order to comply with the current guidelines, the registrant may repeat the unacceptable studies (or upgrade the dominant lethal test) and resubmit for review. Under the revised guidelines, the following will be required as the first tier of studies: 1) Salmonella reverse gene mutation (requirement fulfilled); 2) Mammalian cells in culture forward gene mutation assay, using either the mouse lymphoma L5178Y cells (thymidine kinase locus), CHO or hamster V79 cells (HGPRT gene locus plus an appropriate in vitro test for clastogenicity), or CHO strain AS52 (XPRT locus); and 3) in vivo cytogenetics assay, rodent bone marrow, either metaphase analysis or micronucleus assay. The registrant is also asked to submit a complete bibliography of mutagenicity studies on permethrin, since this will be required for all chemicals under the revised guidelines.

ACTION REQUESTED: ICI Americas submitted four mutagenicity studies for review to support reregistration of permethrin: Salmonella reverse gene mutation assay, dominant lethal test in mouse, mammalian in vivo cytogenetics assay and unscheduled DNA synthesis in rat hepatocytes. All studies except the dominant lethal test were reformatted submissions.

008761

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Linnea J. Hansen, Ph.D. Linnea J. Hansen 9/24/91
Section IV, Tox Branch I (H7509C)
Secondary reviewer: Irving Mauer, Ph.D. 09/23/91 IM 10/29/91
Tox Branch I (H7509C)

DATA EVALUATION REPORT

CHEMICAL: Permethrin Tox. Chem. No.: 652BB

SHAUGHNESSEY NO.: 109701

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

MRID NUMBER: 410311-07

SYNONYMS/CAS No.: 3-(phenoxy) methyl (IRS)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate; Ambush; Permasect; PP557; Ectiban; Indothrin; FMC-33297; CAS# 52645-53-1

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, DE 19897

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Permethrin: An Evaluation in the Salmonella Mutation Assay

AUTHOR: R.D.Callander

STUDY NUMBER: CTL/P/2423 (YV2410)

REPORT ISSUED: February 22, 1989

CONCLUSION(S) - Executive Summary:

Dose levels: 0, 1.6, 8, 40, 200, 1000, 5000 ug/plate in presence and absence of S9.

No increase in revertant colonies was observed among 5 mutant Salmonella strains at any dose level. Some precipitation of the compound occurred at 5000 ug/plate. This study was performed properly and with appropriate controls and is acceptable for regulatory purposes.

Core-Grade: Acceptable
A signed Quality Assurance Statement was present.

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SALMONELLA

A. MATERIALS

1. Test Material: Name: Permethrin
Description: technical, liquid, stable under normal storage conditions and test conditions used in this study):

Batch #: P56;RS/38/F Purity: 95.6% (w/w)
Contaminants: not reported
Solvent used: DMSO

2. Control Materials:

Negative: assay conducted with no solvent or compound additions
Solvent/final concentration: DMSO, 0.1 ml/plate
Positive: Non-activation:
9-Aminoacridine 0.5, 1.0 and2.0 ug/plate TA1537
N-methyl-N'-nitro-N-nitrosoguanidine 1.0, 2.0 and 5.0 ug/plate, TA100, 1535
4-nitro-0-phenylenediamine 1.0, 2.0 and 5.0 ug/plate, TA1538
Daunomycin hydrochloride 0.2, 0.5 and 1.0 ug/plate, TA 98

Activation:
2-Aminoanthracene (2-anthramine) 0.2, 0.5, 1.0 ug/plate (all strains except 1535: 0.5, 1.0 and 2.0 ug/plate

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

Male albino rats (Alderly Park, Alpk:APfSD) treated with a single dose of Arochlor (500 mg/kg body weight) were sacrificed and livers removed aseptically and placed in sterile ice-cold buffer (250 mM sucrose, 50 mM tris base and 1 mM EDTA). A 25% w/w homogenate of liver:buffer was prepared and centrifuged at 4° C at 9000 x g for 10 min. Supernatants were combined, frozen rapidly and stored at -70° C until needed. S9 was assessed for bacterial contamination on nutrient agar plates.

S9 mix was prepared by combining S9 fraction with sterile sucrose-tris-EDTA buffer and a cofactor solution at a 3:7:20 ratio (final concentration of cofactor solution components: 100 mM Na_2HPO_4, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 8 mM $MGCl_2$). Unused mix was discarded at end of the day.

4. Test organisms: S. typhimurium strains

SALMONELLA

___ TA97 _X_ TA98 _X_ TA100 ___ TA102 ___ TA104
_X_ TA1535 _X_ TA1537 _X_ TA1538:

Bacterial strains appeared to have been properly maintained. Frozen cultures used for overnight cultures were prepared from permanent stocks weekly or biweekly. Fresh frozen stocks were tested for histidine requirement and reversion properties using diagnostic mutagens.

Strains were checked for the following genetic markers: DNA repair deficiency, ampicillin resistance, uvrB deletion and deep-rough characters.

5. Test compound concentrations used: 0, 1.6, 8.0, 40, 200, 1000, 5000 ug/plate

Non-activated conditions: For negative strain only controls, 2 plates per strain without S9 were used. Five plates per strain were used for solvent negative controls, with and without S9. Two plates at each of 3 dose levels were used for positive controls. Three plates per dose level and strain were used for the test compound with and without S9 activation. 0.5 ml buffer-cofactor solution was added to nonactivated assays in place of S9 mix.

Activated conditions: Performed as above except for addition of 0.5 ml S9 mix (freshly prepared).

B. TEST PERFORMANCE

1. Type of Salmonella assay:
_X_ standard plate test
___ pre-incubation (___ minutes)
___ "Prival" modification (i.e. azo reduction method)
___ spot test
___ other (describe in a.)

a. Protocol:

Agar plates contained Vogel Bonner minimal medium containing 1.5% w/v agar and 2% w/v glucose. Top agar was prepared with 0.6% w/v agar and 0.5% w/v NaCl to which was added 50 uM biotin and 50 uM histidine.

Overnight cultures of each bacterial strain were prepared and 0.1 ml aliquots placed in sterile plastic bijou bottles. 0.5 ml S9 mix or buffer-cofactor solution were added per strain and dose level to the appropriate bottles and the appropriate test compound/concentration was then added (0.1 ml). 2 ml

(50-53° C) was added to each bottle, mixed quickly and the mixture poured rapidly onto the agar plates. Gelled plates were inverted and incubated at 37° C for 64-68 hrs in the dark.

Plates were examined for microbial contamination and for background lawns and evidence of toxicity. Plates were automatically counted using an AMS 40-10 Image Analyzer (Analytical Measuring Systems, Ltd.). The experiment was repeated twice along with positive and negative controls.

Statistical significance was determined by a one-tailed Student's T-test. Values of $p<0.01$ were considered significant and values between 0.01 and 0.05 considered to indicate possible effects.

2. Preliminary cytotoxicity assay:

There was no mention in the study of preliminary cytotoxicity/dose level testing. However, the test compound was assayed up to the recommended limit test (5000 ug/plate) and over a dose range of 6 dose levels at 5-fold increases, with no mutagenicity evident at any dose level. 5000 ug also appeared to be the solubility limit, as there was significant precipitation of test compound at this dose.

Cytotoxicity was assessed by inspecting the bacterial lawn for inhibition of growth and/or decreased colony formation. For a valid experiment, the lowest test compound should show no evidence of toxicity and at least three test doses should show no significant overt toxicity.

3. Mutagenicity assay:

Results from the experiments are presented in Appendix 1 taken directly from the tables presented in the study.

Control Data is presented in Tables 3 and 4 of the Appendix for Experiments 1 and 2, respectively.

Negative Controls: Within appropriate limits. (+) S9 negative controls had slightly higher numbers of revertants than (-) S9 controls.

Positive Controls: All positive controls showed significant (statistically and biologically) increases in numbers of revertant colonies per plate relative to controls. Revertants increased in a dose-responsive manner.

Test Substance: Permethrin did not appear to be mutagenic under the conditions of this assay in the presence or absence of S9. The experiment as outlined above was repeated twice. In the first experiment (Table 1, Appendix), Strains TA 1538 and TA 100 showed a few statistically significant responses ($p < 0.05$ for TA 1538; $p < 0.01$ pr 0.05 for TA 100) with or without S9 but in each case these differences were at test/control ratios of less than 2. These results were not reproducible in Experiment 2 (Table 2, Appendix). Strain T 100 showed no differences from control and strain TA 1538 showed again a slight response (test/control ratio less than 2). Strain TA 1537 had two values of statistical significance with test/control ratios of 2 or more but since there was no dose response and no reproducibility, they were not considered meaningful.

4. Reviewer's discussion/conclusions:

TB-I agrees with the conclusions of the author that permethrin does not appear to be mutagenic under the conditions in this study. Although some of the test values were statistically significant relative to controls, with a few random exceptions they were all less than 2-fold increases, were not reproducible among the two experiments and are not considered biologically significant. Positive and negative controls all gave appropriate results and positive controls showed dose-responsive increases in number of revertants/plate.

There was no mention in this study of a preliminary dose range-finding and cytotoxicity test. Since the doses spanned a large range (3000-fold difference) up to the compound's solubility limit and since, according to the Assessment of Results section of this study, cytotoxicity was a criteria used to determine validity of the test results, the study is considered acceptable. Information on cytotoxicity (appearance of the bacterial lawns, doses if any at which some effects were observed) should have been provided in the Results/Discussion.

5. No CBI appendix attached

PERMETHRIN

Page ____ is not included in this copy.

Pages 8 through 17 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

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____ Sales or other commercial/financial information.

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Guideline Series 84: **MUTAGENICITY**

Reviewed by: Linnea J. Hansen, Ph.D. 9/23/91
Section IV, Tox. Branch I (H7509C)
Secondary reviewer: Irving Mauer, Ph.D. 09/27/91
Tox Branch I (H7509C)

DATA EVALUATION REPORT

CHEMICAL: Permethrin TOX. CHEM. NO.: 652BB

SHAUGHNESSEY NO.: 109701

STUDY TYPE: Unscheduled DNA Synthesis in Primary Male Rat Hepatocytes

MRID NUMBER: 409436-04

SYNONYMS/CAS No.: 3-phenoxybenzyl-cis-(trans)-3-(2,2 dichloro-vinyl)-2,2-dimethylcyclopropanecarboxylate; PP557, BW0210Z, Ectiban, FMC 33297, Indothrin, NRDC 143, Pramex; CAS No. 52645-53-1

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware 19897

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Chesire SK10 4TK, UK

TITLE OF REPORT: Permethrin: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures

AUTHOR: R.W. Trueman (Study); James Kennelly (Reformat, Summary)

STUDY NUMBER: SV0268 (CTL/P/1888 - CTL Report Number)

REPORT ISSUED: April 26, 1990 (Study completed on 4/12/88)

CONCLUSION(S) - Executive Summary:

Doses tested: Eight doses increasing 10-fold from 10^{-9} to 10^{-2} M (1.174×10^{-6} mg/plate to 11.74 mg/plate).

No evidence of unscheduled DNA synthesis up to 10^{-4} M and possibly 10^{-2} M. Doses above 10^{-5} M to 10^{-2} M (variable, depending upon the experiment) too cytotoxic to assay for UDS. This study was performed properly and is acceptable for

regulatory purposes.

Core-Classification: Acceptable

A signed Quality Asurance Statement was present.

A. MATERIALS

1. Test Material: Permethrin (technical)
Description: straw colored semi-solid. Stable at least 2 years at room temperature.
Batch #: not given, supplied by Imperial Chemical Industries PLC, Plant Protection Division in April, 1987. Given CTL reference number YO040/81/001. Purity 93.5% (w/w)
Contaminants: not reported

Solvent used: DMSO

2. Control Materials:
Negative: Culture media alone
Solvent/final concentration: DMSO, 5 ul/ml
Positive: 6-p-dimethylaminophenylazobenzthiazole (6-BT)

Concentrations: Ten-fold molar dilutions between 10^{-7} and 10^{-4} M
Solvent concentration: 5 ul/ml (50 ul total volume)

3. Test Cells: Primary Male Rat Hepatocytes

Preparation: Male Alderley Park (Alpk:APfSD) rats (Animal Breeding Unit, Imperial Chemical Industries PLC, Pharmaceuticals Division) were anesthetized with Fluothane BP and livers perfused in situ with a Ca^{+2} - free buffer (150 mM NaCl, 3.73 mM $NaHCO_3$, 4.84 mM Na_2HPO_4, 1.24 mM KH_2PO_4, 0.62 mM $MgSo_4$ and 0.62 mM $MgCl_2$, pH 7.4), followed by a second Ca^{+2} - free buffer (142 mM NaCl, 24 mM $NaHCO_3$, 4.37 mM KCl, 1.24 mM KH_2PO_4, 0.62 mM $MgSO_4.7H_2O$, pH 7.4). Collagenase solution (50 mg collagenase and 1 ml 769 mM $CaCl_2.H_2O$) in 10 ml second perfusion buffer) was perfused and recirculated through the liver until cellular dissociation was evident. Livers were excised and minced with scissors, suspended in Williams medium E (WME) and filtered through 159 um nylon mesh. Hepatocytes were sedimented by low speed centrifugation, then resuspended in WME. The centrifugation procedure was repeated twice and viability of isolated hepatocytes was assessed by Trypan blue exclusion.

Maintenance: Dissociated liver cells were plated in WME at 4.5×10^5 cells in 3 cm dishes containing a plastic coverslip for UDS estimation/autoradiography. Cells were incubated for

1.5-2.25 hr at 37° C in humidified 5% CO_2 incubator, re-fed with serum-free WME and used in the UDS test. Triplicate plates were used for each dose level.

Cultures appeared to have been properly maintained.

4. Test compound concentrations used:

Basis for concentrations chosen: To provide a wide dose range including cytotoxic doses (preliminary test not done).

Ten-fold dilutions between 10^{-9} and 10^{-2} M permethrin. Doses at or above 10^{-2}, 10^{-4} and 10^{-5} M, depending upon the experiment, were too cytotoxic to assay cells for UDS.

B. TEST PERFORMANCE

1. Cell treatment:

Cells were incubated in the presence of permethrin at the appropriate concentration and ^{3}H-thymidine at 30 uCi/culture dish (10 uCi/ml media) for 17-20 hr (overnight) at 37°C and 5% CO_2. Cultures were rinsed the next day with Williams incomplete media containing 0.25 mM cold thymidine, then incubated another 24 hr in the same medium. Medium was aspirated and cultures washed in saline, then processed as described below.

2. Cytotoxicity Assay

Preliminary cytotoxicity testing was not performed. A wide dose range (10^{-2} to 10^{-9} M) including cytotoxic doses was used to assess cytotoxicity and only cultures showing no major signs of overt toxicity such as pyknotic nuclei, reduced cell attachment and damaged cells were evaluated for unscheduled DNA synthesis. Four or five dose levels below cytotoxic levels were used for each UDS assay.

3. Autoradiography:

Labelled treated cells attached to the coverslips were washed in normal saline and fixed in a 1:3 acetic acied:ethanol solution. Slides were washed with water, air-dried and mounted on slides. Slides were coated with Ilford emulsion and exposed for 14 days at 4°C in sealed boxes.

Slides were processed by developing in Kodak D19 (1:1 in water), followed by fixation in Ilford HYPAM (1:3 in water)and Meyers Haemalum. Slides were rinsed in water until nuclei turned pale blue, then stained in 1% eosin Y phloxin. They

were washed, dehydrated and cleared, then left 24 hrs before assessment.

4. Scoring:

Slides were coded and nuclear and cytoplasmic counts measured with an automated image analyzer (AMS 40-10). Nuclear and cytoplasmic counts (counts over an adjacent area of cytoplasm the same size as the nucleus) were obtained for at least 25 cells per slide, usually 50 cells per slide, and when possible 100 cells per slide. Darkly labelled nuclei (S phase) were not counted.

5. Evaluation of Results:

A treated culture with mean net grain counts of 5 or more and 20% or more of the cells in repair was considered a positive response. A culture with 0 or fewer mean net grain counts and with less than 20% of the cells was considered a negative response. Any positive responses must also be reproducible.

Results were analyzed statistically to obtain means and standard deviations for each dose level and experiment. The exact method of statistical transformation used was not specified in the study.

C. RESULTS:

The results of the 3 UDS experiments as presented in the study Tables 1-3 are appended to this review.

Cytotoxicity evaluation: There was some variability in cytotoxic response as measured by pyknotic nuclei and damaged cells among the three experiments. While all 3 experiments showed cytotoxicity at the higher dose levels, it was observed only at the highest dose (10^{-2} M) in the first experiment, in doses of 10^{-4} M or higher in the second experiment and at doses of 10^{-5} M or higher in the third experiment, probably due to variable condition of the hepatocytes after isolation and culture. Unscheduled DNA synthesis was not evaluated for these toxic doses.

Unscheduled DNA Synthesis: Permethrin did not appear to cause an increase in unscheduled DNA synthesis above solvent controls at any of the dose levels tested. Mean nuclear counts were lower than cytoplasmic counts for all doses tested. The positive control, 5BT, produced a statistically significant increase in nuclear grain count over the DMSO solvent control (34 - 67, mean nuclear minus mean cytoplasmic counts) and a marked increase in the percent of cells in

repair (91 - 100%).

D. REVIEWER'S DISCUSSION/CONCLUSIONS:

From these studies the authors concluded that permethrin does not induce unscheduled DNA synthesis above negative controls at doses up to cytotoxic levels. The exact cytotoxic dose could not be determined from this study because of variability in toxicity among experiments (10 to 1000-fold difference) but was at least 10^{-4} M based on results from two separate experiments and possibly 10^{-2} M based on a single experiment. The positive control, 6-BT, showed increased net nuclear grain count and % cells in repair. Of the four dose levels of 6-BT tested, only data from 10^{-6} M assays were presented (it would have been useful to see a dose response).

Experimental deficiencies noted in the study are as follows: 1) Cytotoxicity of permethrin was quite variable, depending upon the experiment (10^{-2} to 10^{-5} M). This most likely reflects variability in the condition of the hepatocyte cultures prepared for each experiment. It can probably be safely assumed that permethrin does not cause unscheduled DNA repair up to at least 10^{-4} M since two experiments gave negative results at this concentration. 2) Cells treated with permethrin were assessed for cytotoxicity by visual assessment of pyknotic nuclei and damaged cells instead of the more sensitive trypan blue exclusion method. 3) Quantitative viability data was not presented in the paper for the individual hepatocyte preparations nor for cytotoxicity as measured by relative cell survival at each dose level. However, since the positive controls gave an appropriate positive response, it is assumed that the hepatocyte preparations were within an acceptable viability range. 4) Data for only one dose (10^{-6} M) was presented for the positive control values in each experiment.

Although several deficiencies were identified in this study, TB-I believes that no additional information would be gained by repetition of the experiment and that there is enough information in this study to indicate a negative result for permethrin in the UDS assay.

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UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

APPENDIX

UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

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UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

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Guideline Series 84: **MUTAGENICITY**

Reviewed by: Linnea J. Hansen, Ph.D.
Section II, Tox. Branch I (H7509C)
Secondary reviewer: Irving Mauer, Ph.D.
Section II, Tox Branch I (H7509C)

Linnea J. Hansen 9/24/91
9/23/91
10/1/91

DATA EVALUATION REPORT

CHEMICAL: Permethrin (PP557) TOX. CHEM. NO.: 652BB

SHAUGHNESSEY NO.: 109701

STUDY TYPE: *In vivo* mammalian cytogenetics assay in Rat

MRID NUMBER: 92142-093

SYNONYMS/CAS No.: 3-(phenoxy) methyl (IRS)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate; Ambush; Permasect; Ectiban; Indothrin; FMC-33297; CAS# 52645-53-1

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, DE 19897

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TK, UK

TITLE OF REPORT: 84-2 (b) Mutagenicity Studies: *In vivo* Mammalian Cytogenetics-Bone Marrow Aberrations Test

AUTHOR(S): D. Anderson and C.R. Richardson

STUDY NUMBER(S): SR0004 (Report No. CTL/P/294)

REPORT ISSUED: April 25, 1990 (Reformatted: actual date of study September, 1976)

CONCLUSION(S) - Executive Summary:

Doses tested: 0, 600, 3000 and 6000 mg/kg body weight

Under the conditions of this study permethrin did not appear to induce chromosomal damage up to 6000 mg/kg body wt.

Study deficiencies: No females included in test animals, 8 instead of 10 animals per dose group, no preliminary cytotoxicity data or toxic symptoms at high dose were presented, only one time point was sampled for each assay. The deficiencies in this study are

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sufficient to make it unacceptable for regulatory purposes.

Classification: Unacceptable

A signed Quality Assurance Statement was present.

A. MATERIALS

1. Test Material:
Permethrin (PP 557), technical: straw-colored liquid, stable at room temperature
Batch #: not specified (obtained from Plant Protection Division, Jealotts Hill, Bracknell, Berkshire)
Purity: 94%, 40.3% cis isomer and 59.77 % trans isomer
Contaminants: not specified (no CBI appendix attached)

2. Control Materials:
Negative: test animals with no treatment
Vehicle: none (test compound not administered in vehicle)
Positive: Two positive control chemicals were administered:
Mitomycin C - 2.0 and 3.5 mg/kg body weight
Trimethyl phosphate (TMP) - 3000 mg/kg body wt. (1500 mg/kg for multiple dose regimen).
Both controls administered by intraperitoneal (IP) injection, vehicles if any not specified.

3. Test compound:
Volume of test substance administered: Not specified - variable depending upon dose: ml/kg body wt. = dose (mg/kg body wt)/[specific gravity (g/ml) x 1000 mg/g]. Specific gravity of permethrin = 1.2 g/ml)

Route of administration: Intraperitoneal injection

Dose levels used: 0, 600, 3000 and 6000 mg/kg body wt.

4. Test animals:
a. Species Rat Strain Alderley Park Age 8-10 weeks
Weight: male 150-200 g female not used
Source: not specified (but is most likely from ICI's own animal facilities as for other mutagenicity studies submitted for review).

b. No. animals used per dose: 8 males 0 females

c. Animals were housed in individual mobile cages and given food (Alderley Park rat cubes) and water *ad libitum*. No detail was provided regarding housing conditions (humidity, temperature, etc.).

B. TEST PERFORMANCE

IN VIVO MAMMALIAN CYTOGENETICS

1. Treatment and Sampling Times: Single and multiple dosing regimens used in this study
   a. Test compound- heated to 50-60° C prior to administration
      Dosing: 1) single IP dose - at concentrations described below
      2) Multiple IP dose - five daily doses at concentrations described below

      Sampling (after last dose):
      Single dose: 24 hr
      Multiple dose: 6 hr

   Test compound concentrations used: 600, 3000, 6000 mg/kg

   b. Negative and/or vehicle control
      Dosing: negative control animals received no treatment

   c. Positive controls
      Dosing: single dose: single IP injection
      multiple dose: five consecutive daily injections
      Sampling (after last dose):
      single dose: 24 hr
      multiple dose: 6 hr

   d. Administration of spindle inhibitor: Colchicine, IP injection 2 hrs prior to sacrifice. Dose not specified in report; reference article for technique provided in text (Sugiyama, referenced below).

2. Tissues and Cells Examined:
   X bone marrow

   No. of cells per animal per treatment group examined: 8

   No. cells per animal per control group examined:
   Negative control: 12
   Positive controls: 5

3. Details of cell harvest and slide preparation:

   The procedure for preparation of bone marrow samples was essentially that of Sugiyama (*J. Natl. Cancer Inst.* 47: 1267; 1971). Two hours following injection of colchicine rats were sacrificed by injection of fluothane. Bone marrow cells were removed and cells treated with a hypotonic KCl solution (0.075M), followed by fixation in glacial acetic acid:methanol (1:3). Slides were air-dried and stained with Giemsa, then coded and examined

for chromatid or chromosome gaps, chromatid breaks, fragments, or any other abnormality.

4. Preliminary cytotoxicity assay:

There was no mention of a preliminary cytotoxicity testing. It was stated that the highest dose administered was close to the maximum tolerated dose, but the exact maximum tolerated dose was not given. Observed toxicity symptoms were not described at the highest administered dose.

5. Aberrations assay:

The data summary tables from the study are presented in Appendix I attached to this review. There was no apparent effect of permethrin on chromosomal aberration frequency under the conditions of this study. Mitomycin C gave a positive, dose-related response and TMP also produced a positive response in both experiments. Increases over controls were statistically significant ($p < 0.01$ or 0.001).

6. Statistical Analysis of Data: Statistical methods for transforming and analyzing data are described in Appendix II taken from the study text.

C. REVIEWER'S CONCLUSIONS/DISCUSSIONS:

The authors concluded from this experiment that permethrin did not cause cytogenetic damage under the experimental conditions of this study; however, several experimental deficiencies were noted in this study. Only males were used and there were 8 animals per dose group instead of the recommended 10 (5 male and 5 female). The authors claim that since there are no examples of female-specific mutagens it would be unlikely that permethrin might be a mutagen in females and not males. While such sex-specific responses may indeed be the exception, it cannot be assumed that permethrin is non- mutagenic in both males and females.

It was mentioned that the highest tested dose was close to the maximum tolerated dose but the maximum tolerated dose was not given and toxic symptoms were not described as this dose was approached.

In addition, only one sample time was taken for each dose regimen. The authors claim that the two dose regimens taken together, along with the positive control data, provide sufficient evidence that permethrin does not appear to induce

chromosomal damage. *However, this leaves a large time gap between 30 and 54 hr:* it would be better experimental design to include more than one time point in each experiment to ensure adequate detection of response due to either cell cycle phase-specific and/or phase-lengthening effects. There was also no mention of the solvent/carrier used to administer the positive control substances nor the batch or lot number of the test compound.

For the above reasons the study is considered unacceptable and cannot be used for regulatory purposes.

PERMETHRIN

Page_____ is not included in this copy.

Pages 31 through 32 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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APPENDIX II

Statistics - the data were transformed using a variance stabilising transformation. This also helped reduce the skewedness of the distribution.

The transformation for proportions was used, (Freeman-Tukey, 1950):-

$$y = \sin^{-1}\left(\sqrt{\frac{x}{n+1}}\right) + \sin^{-1}\left(\sqrt{\frac{x+1}{n+1}}\right)$$

where x = number of abnormalities
n = number of cells
y = transformed value.

The data was analysed by an analysis of variance using the following model:-

$$y = \mu + t_i + f_{ij}$$

where μ = overall mean
t_i = treatment effect for the i-th treatment group
f_{ij} = j-th animal effect in the i-th treatment group

A Students' 't' test (one-sided) was used on transformed values.

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Linnea J. Hansen, Ph.D. Linnea J. Hansen 10/24/91
Section IV, Tox Branch I (H7509C)
Secondary reviewer: Irving Mauer, Ph.D. Irving Mauer 10/24/91
Tox Branch I (H7509C)

DATA EVALUATION REPORT

CHEMICAL: PP 557 TOX. CHEM. NO.: 652BB

SHAUGHNESSEY NO.: 109701

STUDY TYPE: Dominant Lethal Test in Rodent

MRID NUMBER: ~~00043730~~ 40436-04

SYNONYMS/CAS NO.: Permethrin; 3-(phenoxy) methyl (IRS)-cis, trans-3-(2,2- dichloroethenyl)-2,2-dimethylcyclopropane carboxylate; Ambush; Permasect; Ectiban; Indothrin; FMC-33297; CAS# 52645-53-1

SPONSOR: Imperial Chemical Industries, Alderley Park, Macclesfield, Cheshire, UK

TESTING FACILITY: Inveresk Research International, Edinburgh, EH21 7UB, Scotland

TITLE OF REPORT: Dominant Lethal Study in Mice of ICI-PP 557

AUTHORS: D.B. McGregor, G.A. de S. Wickramaratne

STUDY NUMBER: 406722

REPORT ISSUED: November, 1976

CONCLUSION(S) - Executive Summary:

Doses administered: 0, 15, 48 and 150 mg/kg body wt/day; five consecutive daily doses administered orally to male mice.

No evidence of increased early fetal death above the negative control at any dose level up to 8 weeks post-treatment. The positive control, ethylmethanesulphonate, induced a statistically significant increase in early fetal deaths.

This study was not properly conducted according to guideline requirements and is not acceptable for regulatory purposes.

SALMONELLA

Classification: Unacceptable (can be upgraded)

A signed Quality Assurance Statement was not included.

A. MATERIALS

1. **Test Material:** PP 557 (permethrin), technical; straw-colored solid, low melting point, stable at room temperature
Batch #: 25, I.C.I. Ltd.
Purity: 95.3%, cis:trans ratio 37.5:57.8
Contaminants: not specified (CBI Appendix is not attached).

2. **Control Materials:**
Negative (vehicle): corn oil
dose: 10 ml/g body wt.
administered: orally for 5 days
Positive: Ethylmethanesulphonate
dose: 100 mg/kg body wt. in water
administered: orally for 5 days

3. **Test Animals:** Species mouse Strain CD-1 Age 8 - 10 wks
Weights of males and females not specified.
Source: Charles River (location of supplier not specified)

4. **Test compound concentrations:** 15, 48 and 150 mg/kg body wt.

B. TEST PERFORMANCE

1. **Dose Level Determination:**

Dose levels for this study were selected based upon results from a range finding study. 6 male mice per group received 5 daily oral doses of PP 557 at the doses listed below and survival after 7 days was determined. 150 mg/kg body wt was chosen as the high dose based upon survival (see below). No body weight data was provided.

mg PP 557/kg/day	18.8	37.5	75	150	300	600
Survival at 7 days	6	6	6	4	4	4

2. **Treatment:**

Five groups of 15 male mice were treated orally at the doses described (Section A-4) above for 5 consecutive days. Males were mated with untreated females immediately following last dose.

3. Mating:

Male mice were preselected for fertility in mating trials. 75 fertile males were selected for use in the dominant lethal test. Immediately following treatment as described above, males were caged with 2 virgin female mice (8-10 weeks old) every 7 days for a total of 8 weeks (total 16 female mice/male). It was assumed that matings occurred by 2-3 days after cohabitation. Females were sacrificed 13 days after the estimated date of fertilization. Males were sacrificed at the end of the 8 week mating period.

4. Cesarian Procedures:

Uteri of sacrificed females were examined for 1) live implantations, 2) early deaths and 3) late deaths. Any abnormalities observed were recorded.

5. Statistical Analysis:

A hierarchical analysis of variance was applied to the data using the formula below:

$$Y_{ijk} = u + t_i + m_{ij} + f_{ijk}$$

Y = early deaths
i = ith treatment (t) group
j = jth male (m) within a group
k = kth female (f) (1 or 2) within a male
u = general mean

Data were analyzed using a Genstat program and variance was stabilized using the transform $\sqrt{Y} + \sqrt{Y + 1}$.

C. RESULTS:

1. Clinical Symptoms:

There was no mention in this study of clinical symptoms or weight loss during treatment or mating. Female mice showed increased mortality during the course of the study but no cause was determined. There was no increase in male mortality with increased test compound dose. Treatment with PP 557 did not appear to cause decreased fertility in males under the conditions of this study.

2. Pregnancy Frequency:

Table 1 below presents pregnancy rates for treatment groups during each week.

TABLE 1: PREGNANCY RATE[1]

WEEK	# PREGNANT/TOTAL # FEMALES (% PREGNANT)				
	NEGATIVE CONTROL	POSITIVE CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1	24/30(80)	27/30(90)	28/30(93)	27/30(90)	26/30(87)
2	25/28(89)	25/29(86)	25/29(86)	27/27(100)	23/30(77)
3	30/30(100)	25/30(83)	29/30(97)	26/28(93)	25/30(83)
4	28/30(93)	28/30(93)	26/26(100)	27/28(96)	25/26(96)
5	26/30(87)	29/30(97)	25/26(96)	25/28(89)	27/30(90)
6	30/30(100)	29/30(97)	26/26(100)	23/26(88)	30/30(100)
7	28/30(93)	30/30(100)	24/26(92)	23/26(88)	27/30(90)
8	29/30(97)	29/30(97)	25/26(96)	23/24(96)	25/27(93)
MEAN %	92.4	92.9	95.0	92.6	89.3

1 Data taken from Table 1 of study

No apparent decrease in pregnancy rate was observed among females mated with treated or positive control males. Control females showed an overall mean pregnancy frequency of 92.4% compared to 95, 92.6 and 89.3% at low, medium and high doses, respectively.

3. Cesarian Data:

Total implantations/pregnancy: Table 2 below presents implantation rates for each treatment group during the study.

TABLE 2: MEAN # IMPLANTATIONS/PREGNANCY[1]

WEEK	NEG. CONTROL	POSITIVE	LOW DOSE	MID DOSE	HIGH DOSE	F^2
1	11.19	11.04	11.71	11.50	11.80	0.54
2	12.37	12.40	12.89	12.89	13.31	0.84
3	13.13	12.61	11.63	13.62	12.32	2.53*
4	13.23	12.77	12.88	12.11	12.89	1.06
5	12.54	13.03	12.88	13.04	12.70	0.28
6	12.10	12.50	12.50	13.33	12.10	1.70
7	12.43	12.90	11.50	11.83	12.53	2.71*
8	12.07	12.50	12.47	12.17	11.81	0.46

1 Data taken from Table 2 of study
2 F = Variance ratio
* $p < 0.05$

Females mated with low dose males showed statistically significant ($p < 0.05$) decreases in total implantations during

Weeks 4 and 7. Since these decreases were sporadic and not dose-related, they were not considered treatment-related. No other significant differences were observed.

Early Deaths:

Early death results were expressed in several ways. Pregnancies with 1 or more early deaths and 2 or more early deaths are shown below in Table 3.

ABLE 3: # PREGNANCIES (%) WITH ≥ 1 OR ≥ 2 EARLY DEATHS[1]

	NEG. CONTROL		POS. CONTROL		LOW DOSE		MID DOSE		HIGH DOSE	
WEEK	≥ 1	≥ 2	≥ 1	≥ 2	≥ 1	≥ 2	≥ 1	≥ 2	≥ 1	≥ 2
1	9(38)	2(8)	23(85)	19(70)	12(43)	5(18)	8(30)	1(4)	13(50)	4(15)
2	8(32)	2(8)	19(76)	11(44)	8(32)	0(0)	3(11)	0(0)	13(57)	3(13)
3	12(40)	6(20)	13(52)	4(16)	11(38)	4(14)	15(58)	5(19)	15(60)	5(20)
4	11(39)	2(7)	12(43)	4(14)	15(58)	8(31)	16(59)	5(19)	10(40)	5(20)
5	13(50)	6(23)	10(34)	1(3)	7(28)	3(12)	12(48)	3(12)	12(44)	4(15)
6	12(40)	5(17)	13(45)	3(10)	12(46)	4(15)	10(48)	2(7)	9(30)	3(10)
7	17(61)	3(11)	2(7)	0(0)	8(33)	5(21)	7(30)	3(13)	15(56)	3(11)
8	7(24)	3(3)	12(41)	2(7)	6(24)	2(8)	7(30)	1(4)	8(32)	3(12)

Data taken from Tables 3 and 4 of study

Early deaths were increased in the positive control group during Weeks 1 and 2. Early deaths per pregnancy were quite variable among all control and treatment groups when expressed as 1 or more early deaths/pregnancy; however, when data was expressed as 2 or more early deaths/pregnancy, this variability was reduced (at the possible risk of underestimating dominant lethal effects) and only the positive control group showed distinct increases. No statistical treatment of this data was performed in the study.

The number of live implants and late deaths per pregnancy are shown below in Table 4. The authors chose to present this data because dominant lethal agents may cause both pre-implantation losses and early deaths and small effects in both should be detectable using this statistic.

TABLE 4: LIVE IMPLANTS AND LATE DEATHS PER PREGNANCY[1]

WEEK	NEG. CONTROL	POS.CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	F[2]
1	10.69	8.86	11.11	11.18	11.11	4.14**
2	11.93	10.43	12.54	12.79	12.46	4.51**
3	12.40	11.75	11.13	12.77	11.32	2.03
4	12.57	12.23	11.92	11.32	12.23	1.41
5	11.68	12.60	12.50	12.46	12.03	0.78
6	11.47	11.87	11.88	12.70	11.67	1.25
7	11.67	12.83	10.92	11.42	11.87	4.09**
8	11.80	12.00	11.96	11.75	11.38	0.32

1 Data taken from Table 5 of study
2 F = Variance ratio
** $p < 0.01$

Positive controls showed statistically significant decreases in mean live implants and late deaths per pregnancy compared to negative controls during Weeks 1 and 2. According to the study authors, the variance during Week 7 was primarily due to the high number of live implants and late deaths among positive controls rather than a reduction in the treated groups (the reduction in low dose animals at Week 7 was not statistically significant compared to negative controls).

Early deaths expressed as % of total implants are presented below in Table 5.

TABLE 5: EARLY DEATHS - % OF TOTAL IMPLANTS[1]

WEEK	NEG. CONTROL	POS. CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1	4.79	20.13	5.18	2.78	6.17
2	3.50	15.58	2.49	0.86	5.96
3	5.58	6.03	4.41	6.21	7.14
4	5.14	4.48	7.46	5.40	5.25
5	6.48	3.17	3.11	4.66	5.33
6	5.23	4.93	4.92	4.59	3.58
7	5.98	0.52	5.07	3.66	5.65
8	2.30	3.87	3.85	2.85	3.74
MEAN %	4.90	6.82	4.57	4.03	5.32

1 Data taken from Table 6 of study

The only marked increases in early deaths as % of total implants occurred in the positive control group during Weeks 1 and 2. Extremely small values were observed among mid dose animals in Week 2 and among positive control animals, Week 7. No statistical treatment was presented for this data.

The number of statistically transformed early deaths per pregnancy for each group is presented in Table 7. Data was transformed to stabilize variance before analysis.

TABLE 6: TRANSFORMED EARLY DEATHS/PREGNANCY[1]

WEEK	NEG. CONTROL	POS. CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	F[2]
1	1.59	3.05	1.74	1.43	1.90	12.94***
2	1.54	2.83	1.51	1.15	2.00	15.62***
3	1.79	2.00	1.62	2.00	2.13	1.29
4	1.71	1.66	2.08	1.99	1.73	0.94
5	1.98	1.56	1.47	1.74	1.80	1.54
6	1.72	1.77	1.77	1.75	1.52	0.37
7	1.97	1.09	1.65	1.50	1.85	5.92***
8	1.35	1.66	1.46	1.53	1.52	0.51

1 Data taken from Table 7 of study
2 F = variance ratio
*** $p < 0.001$

Statistically significant differences observed during Weeks 1 and 2 were due to increased early deaths in the positive control group relative to the negative control group. The statistically significant differences observed during Week 7 were due to a decrease in the positive control group relative to the negative control. Treatment with PP 557 did not appear to cause an increase in dominant lethal effects.

D. REVIEWER'S DISCUSSION/CONCLUSIONS:

The study authors concluded that PP 557 did not cause an increase in heritable genetic defects in mice under the conditions of this study. Litters sired by males treated with ethylmethanesulphonate, the positive control, showed a statistically significant increase in early deaths of fetuses at Weeks 1 and 2 but not at later times.

Deficiencies noted in this study include: 1) no evidence of systemic toxicity (clinical symptoms or body weight decrease) or target organ toxicity (decreased fertility) in males treated with 150 mg/kg/day in the primary study. The range finding study used mortality (2/6 animals) as criteria for toxicity but body weight was not mentioned. There was no dose-related increase in mortality in the range finding study at 300 or 600 mg/kg/day as might be expected if the deaths were truly treatment-related; 2) no statistical treatment of some data. There was also no Quality Assurance Statement and the copy of the study received for review was barely legible to illegible in places.

DOMINANT LETHAL TEST IN RODENT

This study is graded unacceptable based on insufficient proof that the high dose (150 mg/kg/day) provides a sufficient dose to test dominant lethal effects. It can be upgraded if the registrant can provide evidence of systemic toxicity and transport to the target tissues (seminiferous tubules) in effective concentrations in the high dose males, or evidence that PP 557 does not reach the target organ (testes) in mice because of the blood-testicular barrier. Statistical treatment of all data is also requested.

HANSEN/PC-1/DER.DLT/PERMETHRIN DOM LETHAL/9-28-91/0006/1-2252