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

MEMORANDUM

JUL 20 1986

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 6F3318. Karate 1E. Petition for Tolerance of PP321 on Cotton

Tox. Chem. No. 725C
Related Tox. Chem. No. 271F
Project No. 956

TO: George LaRocca (PM Team #15)
Registration Division (TS-767c)

FROM: Pamela M. Hurley, Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

William Burnam, Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769c)

Background:

ICI Americas Inc. is requesting a permanent tolerance for PP321 on cotton. This pesticide chemical is an ingredient of the insecticide, Karate 1E. PP321 is also one of two enantiomeric pairs which comprise the pesticide, cyhalothrin (PP563). The formulation, Karate 1E will be used as a foliar spray. The manufacturer recommends application every 5 to 7 days or as needed using either a minimum of 1.5 gallons per acre by air or a minimum of 5 gallons per acre by ground. No more than 3 pints (0.375 lb ai) per acre per season is to be applied.

ICI has provided subchronic, acute and metabolism data on both PP321 and cyhalothrin to support the use of the chronic studies that have been conducted on cyhalothrin as partially fulfilling the toxicity data required for the tolerance petition on PP321.

The substance identification and technical data for PP321 and Karate 1E are given in a previous Experimental Use Permit Petition (10182-EUP-UR), memorandum to George LaRocca, dated May 8, 1986.

Comments:

1. The following toxicity studies are required to be submitted in support of the tolerance petition (preceded by a (*)). Additional studies required for registration of the product are preceded by a (**) (ref. Fed. Reg. 40 CFR Part 158, October 24, 1984).

Technical Product	Required	Satisfied
**Acute oral LD_{50}	Yes	Yes
**Acute dermal LD_{50}	Yes	Yes
*90-day feeding studies rodent & nonrodent	Yes	Yes (comment 2)
**21-day dermal	Desirable	Pending (comment 5)
*Chronic feeding rodent & nonrodent	Yes	Yes (comments 2,3)
*Oncogenicity - rat & mouse preferred	Yes	Yes (comment 2)
*Teratogenicity - 2 species	Yes	Yes (comment 2)
*Reproduction, 2 generation	Yes	Yes (comment 2)
*Mutagenicity		
Gene mutation	Yes	Yes
Struct. chrom. aberration	Yes	Yes
Other genotoxic effects	Yes	Yes
End Use Product		
**Acute oral LD_{50}	Yes	Yes
**Acute dermal LD_{50}	Yes	Yes
**Acute inhalation LC_{50}	Yes	Yes
**Primary eye irritation	Yes	Yes
**Primary dermal irritation	Yes	Yes
**Dermal sensitization	Yes	Yes
Pure Active Ingredient		
*General Metabolism	Yes	Yes (comments 2,4)

2. ICI has requested that the long term studies conducted on cyhalothrin be used in partial fulfillment of the toxicity data required for the tolerance petition for PP321. PP321 consists of 2 of the 4 enantiomers of cyhalothrin. On the basis of structural considerations and metabolism and subchronic data on both PP321 and cyhalothrin, the Toxicology Branch accepts the long term data on cyhalothrin as partial fulfillment of the toxicity studies required for the tolerance petition on PP321.

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3. The chronic dog study was only for six months. The Toxicology Branch is accepting this study in fulfillment of the required nonrodent chronic feeding study because it was completed prior to the publication of the Subpart F guidelines.

4. Extensive metabolism studies have been conducted on the purified form of cyhalothrin. A comparative study between cyhalothrin and PP321 has indicated that their absorption, distribution, metabolism and excretion patterns are identical following a single 1 mg/kg dose in the male rat. Therefore, the Toxicology Branch is accepting the metabolism studies conducted on cyhalothrin along with the comparison study mentioned above in fulfillment of the metabolism studies required for the tolerance petition for PP321.

5. The 21-day dermal study in rabbits was classified as core supplementary because of the possibility that the animals had coccidiosis. This petition will not be held up because of the data gap in this area. However, The Toxicology Branch has already requested additional slides from this study in a previous memorandum (EUP Petition 53218-EUP-1,2) and that the slides be submitted for evaluation.

6. The inert ingredients in the product Karate 1E have been cleared for use under 180.1001.

7. The draft label (10/85) should be changed to fit with toxicity category I because the dermal irritation category is corrosive. In addition, the precautionary statement should reflect that the formulation could be corrosive to the skin. The registrant should also include a statement that the formulation is a potential sensitizer.

8. A copy of the proposed tolerances (Section F) is attached.

9. The Toxicology Branch has no objection to granting the petition for a permanent tolerance for PP321 on cotton, once the label has been modified. An 8-point document is attached.

SECTION F

PROPOSED TOLERANCES

It is proposed that tolerances be established for residues of (±)-alpha-cyano-(3-phenoxyphenyl)methyl(±)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-2-enyl)-2,2-dimethylcyclopropanecarboxylate in or on the following raw agricultural commodities:

Cattle, fat	0.01	parts per million
Cattle, meat	0.01	
Cattle, meat by-products	0.01	
Cottonseed	0.01	
Goats, fat	0.01	
Goats, meat	0.01	
Goats, meat by-products	0.01	
Hogs, fat	0.01	
Hogs, meat	0.01	
Hogs, meat by-products	0.01	
Horses, fat	0.01	
Horses, meat	0.01	
Horses, meat by-products	0.01	
Milk	0.01	
Sheep, fat	0.01	
Sheep, meat	0.01	
Sheep, meat by-products	0.01	

8-Point Review

[Prepared for 6F3318, PP321 on cotton, May, 1986]

1. Toxicity data with technical grade PP321 and with technical grade cyhalothrin (justification given in point #8 of this document) considered in support of this tolerance (selected studies).

Acute oral LD_{50}, rats PP321	79 mg/kg in males 56 mg/kg in females
90-day feeding, rats PP321	NOEL 50 ppm, LOEL 250 ppm based on reduced body wt gain
26-week oral, dogs Cyhalothrin	NOEL 1 mg/kg/day LOEL 2.5 mg/kg/day (liquid feces)
Chronic feeding, rat Cyhalothrin	NOEL 50 ppm, LOEL 250 ppm (reduced body wt gain. No onco. effects)
Chronic/Onco, mouse Cyhalothrin	NOEL 100 ppm, LOEL 500 ppm (decreased body wt gain. No onco. effects)
Teratology, rabbit Cyhalothrin	NOEL maternal tox. 10 mg/kg/d, LOEL 30 mg/kg/d (decreased body wt gain). NOEL fetotox. 30 mg/kg/d Not teratogenic.
Teratology, rat Cyhalothrin	NOEL maternal tox. 10 mg/kg/d, LOEL 15 mg/kg/d (reduced body wt). NOEL embryo-leth. & fetotox. 15 mg/kg/d. Not teratogenic.
Reproduction - 3 gen., rat Cyhalothrin	NOEL parental tox. 10 ppm, LOEL 30 ppm (decr. bw gain). Offspring: NOEL 10 ppm, LOEL 30 ppm (decreased bw gain).

Metabolism, rats Cyhalothrin and PP321	55% oral absorption. Extensively metabolized when absorbed; cleavage of ester to cyclopropylcarboxylic acid & phenoxybenzyl derivatives. Accumulation of unchanged cmpd. in fat upon chronic administration.
Mutagenicity - Ames Gene Mutation (PP321)	Not mutagenic
Mutagenicity - Chrom. Aberr. in rodents (PP321)	Did not induce micronuclei
Mutagenicity - Gene mutation in Lymphoma cells (PP321)	Not mutagenic
Mutagenicity - In Vitro Cytogenetics (PP321)	Not a clastogen in human lymphocytes

2. Additional toxicity data considered desirable:
None

3. Not applicable

4. This is a new pesticide. No other tolerances have been granted.

5. Establishing this tolerance will theoretically contribute 0.0059 mg/day to the diet (1.5 kg) and will result in 1.98% of the MPI being used up (see computer printout, next page).

6. The 3-generation reproduction study on cyhalothrin in the rat with a safety factor of 100 was used to calculate the ADI. The NOEL was 0.5 mg/kg/day (10 ppm). The ADI is calculated to be 0.0050 mg/kg/day and the MPI is 0.3000 mg/day (60 kg).

7. There are no pending regulatory actions against registration of the pesticide.

8. The registrant has requested that the long term studies conducted on cyhalothrin be used in partial fulfillment of the toxicity data required for the tolerance petition for PP321 (Karate). PP321 consists of 2 of the 4 enantiomers of cyhalothrin. On the basis of structural considerations and metabolism and subchronic data on both PP321 and cyhalothrin, the Toxicology Branch (TB) accepts the long term data on cyhalothrin as partial fulfillment

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of the toxicity studies required for the tolerance petition on PP321. TB has also decided that both cyhalothrin and PP321 will be considered to be the same chemical for the purpose of establishing the ADI and TMRC (see attached memorandum from R. Engler to Pam Hurley, dated July 10, 1986). Any future tolerance petitions for either cyhalothrin, PP321 or any other mixtures of the 16 possible isomers of the chemical structure (provided that the appropriate toxicological data are provided) will be treated as if they are the same chemical and the proposed tolerances will be added to the percent ADI calculated for PP321 in this action.

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NO CFR NUMBER

Cynalothrin/ PP321 (Karate) 4/23/86

Unverified Printout

ACCEPTABLE DAILY INTAKE D

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NAT,Older NOEL mg/kg	ppm	p.f.	ADI mg/kg/day	PPI mg/day(60kg)
0.500	10.00	100	0.0050	0.3000

Current Action 6F3316

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Cattle(26)	0.010	7.16	0.0108
Milk&Dairy Products(93)	0.010	2.42	0.00429
Goats(62)	0.010	0.03	0.00005
Hogs(69)	0.010	3.43	0.00052
Horses(206)	0.010	0.03	0.00006
Sheep(145)	0.010	0.19	0.00063
Cottonseed (Oil)(41)	0.010	0.15	0.00002

PPI	MRID	% PPI
0.3000 mg/day(60kg)	0.0059 mg/day(1.5kg)	1.96

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JUL 10 1986

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: ADI/RfD for Cyhalothrin and Karate
Caswell Nos. 271F and 725C

FROM: Toxicology Branch ADI Committee

TO: Pam Hurley
Section II, Toxicology Branch/HED (TS-769)

and

Residue Chemistry Branch/HED (TS-769)

Background:

The RfD/ADI documents for these "two" chemicals were presented to the Toxicology Branch ADI Committee. The following facts were presented to the Committee:

1. Cyhalothrin and Karate are basically the same chemical, the differences are found in their stereo chemistry.

2. Cyhalothrin consists of four (4) stereo isomers and Karate of two (2). The two Karate isomers are contained in Cyhalothrin, they represent 40% of Cyhalothrin.

3. At present, it appears that the use of Cyhalothrin focuses on using it on cattle (meat and milk tolerances) and Karate is intended for food crops (rac tolerances).

4. The major studies supporting an ADI/RfD were performed on Cyhalothrin, but the registrants intend to use these studies in support of either chemical.

5. While there might be some difference between the two "chemicals" especially with respect to efficacy, 90-day studies in rats have shown that there is no significant difference in their biological effects on mammals.

6. The reproduction study (3 generation) for Cyhalothrin shows the most sensitive toxicological endpoint (NOEL = 0.5 mg/kg/day).

Options: Essentially three options were discussed.

1. To establish an ADI for Cyhalothrin at 0.005 mg/kg/day (NOEL/100) and to establish a separate ADI for Karate at 0.002 mg/kg/day (NOEL/100/2.5) accounting for the fact that only 40% of the Cyhalothrin fed was actually Karate.

2. To establish an ADI on Cyhalothrin (as option 1) and require all the long-term data on Karate to establish a separate ADI.

3. To establish an ADI for Cyhalothrin/Karate based on the Cyhalothrin data (i.e., 0.005 mg/kg/day).

Consensus:

The consensus of the ADI committee was to use option 3 for the following reasons:

(1) All information, particularly the 90 day rat studies, show that there is no significant difference in the toxicity of the different stereo isomeric mixtures of this chemical.

(2) Establishing two ADIs for essentially the same chemical would provide the opportunity to expose the population to excessive levels of the Cyhalothrin/Karate complex, especially under the present use practices where meat and milk tolerances would be evaluated against the "Cyhalothrin ADI" and other tolerances against the "Karate ADI."

(3) Separate tolerances for stereo-isomers of the same chemicals would be inconsistent with the practice of setting combined tolerances on salts, esters and acids of the same chemical; the basic toxicological properties remain the same even though these are not identical chemicals, in the strictest sense.

(4) To prorate the combined Cyhalothrin/Karate ADI/RfD by a factor of 0.4 was not considered necessary since comparative toxicity tests did not show differences which would support this type of amortization.

(5) Referral to RCB: The committee, as a result of the above consensus concluded that residue evaluations and expressions for either Cyhalothrin or Karate must include any and all stereo isomers.

KARATE TOXR 005316

Page_____ is not included in this copy.

Pages 11 through 14 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.

X A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ 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.

Studies Reviewed

Karate

.....Technical.....

Study	Results	Core Classification
Mutagenicity - Ames - Gene Mutation	Not mutagenic under conditions of assay	Acceptable
Mutagenicity - In Vitro Cytogenetics	Not clastogenic under conditions of assay	Acceptable
Mutagenicity - Gene Mutation in Lymphoma Cells	Not mutagenic in mouse lymphoma cells	Acceptable
Mutagenicity - Chromosome Aberration in Mouse Micronucleus	Did not induce micronuclei under conditions of study	Acceptable
Subchronic Oral - rat	NOEL 50 ppm & LOEL 250 ppm based on reduced bw gain	Guideline
Metabolism - Comparative Absorption with cyhalothrin & R157836	Absorp., Distrib., Excret., Metab. ident. following single 1 mg/kg dose	Guideline with previous studies

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Studies Reviewed

Cyhalothrin

.....Technical.....

Study	Results	Core Classification
28-Day Feeding	250 ppm induced SER proliferation, incr. APDM activity (reversible) & decr. bw gain	Acceptable for purpose of study
28-Day Feeding Comparison with PP564	PP563:Males LOEL 20 ppm Females NOEL 20 ppm (lowest dose). PP564 less toxic. Clinical signs of neuro-toxicity, decreased body-weight gain.	Acceptable for purpose of study
Metabolism - Bioaccumulation in rat	Slowly taken up & slowly released by fat. Quickly released by blood, liver, kidneys.Rate of metab. for both enantiomer pairs ident.	Guideline with previous studies

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

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STUDY TYPE: Mutagenicity (84-2) - Gene Mutation in Bacteria (Ames)

TOX. CHEM. NO.: 725C

ACCESSION NUMBER: 073981

TEST MATERIAL: [(RS)-alpha-cyano-3-phenoxybenzyl,(1 RS),cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate]

SYNONYMS: PP321, Karate (name of formulation), constituent of cyhalothrin or PP563

STUDY NUMBER(S): YV1309

REPORT NUMBER: CTL/P/1000

SPONSOR: ICI Plant Protection Division, Fernhurst, Haslemere, Surrey, UK

TESTING FACILITY: ICI Cntrl. Tox. Lab, Alderly Park, Macclesfield, Cheshire UK

TITLE OF REPORT: PP321 - An Evaluation in the Salmonella Mutagenicity Assay

AUTHOR(S): RD Callander

REPORT ISSUED: July 12, 1984

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 16C

CONCLUSION: PP321 was not mutagenic under the conditions of the assay.

Classification: Acceptable

MATERIALS AND METHODS:

Chemical:

PP321 was given the following reference numbers: CTL - YO2537/001/005, Plant Protection # SC47/83 and batch reference P13 (D3239/11). The stated purity was 96.5% w/w.

Protocol:

PP321 was assayed twice in 5 Salmonella typhimurium Ames strains at the following dose levels: 1.6, 8.0, 40, 200, 1000 and 5000 micrograms/plate, both with and without metabolic activation (S-9 mix prepared from Aroclor 1254-induced Sprague-Dawley rats). The five tester strains used were TA 1535, TA 1537, TA 1538, TA 98 and TA 100. The positive controls used for the test were 2-aminoanthracene (with S-9 mix for all strains), N-methyl-N'-nitro-N-nitrosoguanidine (without S-9 mix for TA 1535 and TA 100), 2-methoxy-6-chloro-9-(3(2-chloroethyl)aminopropylamino) acridine.2 (without S-9 mix for TA 1537), 4-nitro-o-phenylenediamine

(without S-9 mix for TA 1538) and Daunorubicin (without S-9 mix for TA 98). Both solvent and 'absolute' negative controls were included in the test. Revertant colonies were counted using an electronic colony counter calibrated to count standard plates, except in some cases where precipitation of the test chemical at high doses required manual counting. The incubation period was 64-68 hours at 37°C. A positive response was considered to be a two-fold or greater increase in the mean number of revertant colonies per test plate over and above the mean number of revertant colonies for controls. A second criterion for a positive response was a statistically significant dose-related increase in the number of revertants. Two separate tests were conducted.

RESULTS:

In both experiments, PP321 did not induce a significant positive response in the presence of S-9 mix. In experiment 1, a slight increase in the number of revertant colonies was observed in 1 dose level in tester strains TA 1537 (1000 micrograms/plate) and TA 1538 (5000 micrograms/plate) without S-9 mix, but the increase did not exceed 1.5x the background rate (statistical significance between 0.05 and 0.01). In experiment 2, these slight increases were not present. The negative control data was within an acceptable range (authors of report and Environ. Mutagen. 1:87-92. 1979) and the positive control data showed appropriate dose-related positive responses (1.8 - 181.0 fold over controls depending upon strain and chemical). PP321 is considered to be non-mutagenic in this assay.

DISCUSSION:

The compound precipitated at the two highest dose levels: 5000 ug/plate (all plates in experiment 1, (-)S-9 plates in experiment 2) and 1000 ug/plate ((-)S-9 plates in experiment 1), indicating a limit of solubility of the test compound in the assay system.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

005316

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (84-2) - Chromosomal Aberrations in Rodents (Mouse Micronucleus)

TOX. CHEM. NO.: 725C

ACCESSION NUMBER: 073981

TEST MATERIAL: [(RS)-alpha-cyano-3-phenoxybenzyl,(1 RS),cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate

SYNONYMS: PP321, Karate (formulation), constituent of cyhalothrin + PP563

STUDY NUMBER(S): SM0155

REPORT NUMBER: CTL/P/1090

SPONSOR: ICI Plant Protection Division, Fernhurst, Haslemere, Surrey UK

TESTING FACILITY: ICI Cntrl. Tox. Lab, Alderly Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: An Evaluation of PP321 in the Mouse Micronucleus Test

AUTHOR(S): Sheldon T, Richardson CR, Shaw J, Barber G

REPORT ISSUED: October 31, 1984

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab Reference 17C

CONCLUSION: PP321 did not induce micronuclei under the conditions of the study.

Classification: Acceptable

MATERIALS AND METHODS:

Chemical:

PP321 was supplied by ICI PLC, Plant Protection Division, Fernhurst, UK. It was a pale grey/off white solid with a purity of 96.5% w/w PP321. The reference numbers were: Plant Protection Division # SC46/83 and CTL # Y0537/001/012. Cyclophosphamide was used as a positive control in this study. It was supplied by Ward Blenkinsop and Co. Ltd., London and was given the CTL reference number Y00790/001.

Animals:

Male and female C57Bl/6J mice were supplied by the Animal Breeding Unit, Alderly Park, Macclesfield, Cheshire, UK. They were 8-12 weeks old.

Protocol:

The study was divided into 2 parts: determination of Median Lethal Dose (MLD) and the micronucleus test itself.

MLD Determination - Phase I

Groups of 5 male and 5 female were administered a single intraperitoneal injection of the test compound at the following dose levels: 5, 25, 50, 75 and 100 mg/kg. The animals were observed daily for deaths over a seven day period.

Dosing of Animals for Micronucleus Test - Phase II

Sixty animals of each sex were divided into 4 groups of 15: 2 treated, 1 negative control and 1 positive control. The test animals were given a single i.p. dose of PP321, using doses equivalent to 80% or 50% of the MLD/7 (35 mg/kg and 22 mg/kg respectively). The negative controls received 10 ml/kg corn oil and the positive controls received 65 mg/kg cyclophosphamide. Five animals of each sex from each group were killed by cervical dislocation at 24, 48 or 72 hours and the femurs were removed. Slides of bone marrow were prepared and stained with polychrome methylene blue and eosin. Five hundred polychromatic erythrocytes were examined and the number containing micronuclei were scored. The samples were also examined for evidence of cytotoxicity. The results were analyzed statistically using a one-sided Student's 't' test.

RESULTS:

There was a slight error in calculation of the MLD/7. The animals should have been dosed 40 and 25 mg/kg instead of 35 and 22 mg/kg. However, the doses given were sufficiently high to give a cytotoxic effect at both dose levels. There was a statistically significant reduction in the ratios of polychromatic erythrocytes to mature erythrocytes in the treated animals at both dose levels 48 hours (but not at any other time points) after dosing when compared to controls. No statistically significant increases in the frequency of micronuclei were observed at any dose level of PP321 at any of the 3 sampling times. The positive control exhibited significant increases in micronuclei at 24 and 48 hours ($P<0.01$, up to 27.8 micronuclei/1000 cells), but by 72 hours the incidence of micronuclei had dropped back to control levels (approx. 4-5 micronuclei/1000 cells).

DISCUSSION:

Although in the treated animals there was a decrease in the ratio of polychromatic erythrocytes to mature erythrocytes when compared to controls at 48 hours, there is still some question as to whether or not the test chemical reached the target organ because at this time period the control values were quite a bit higher than they were at 24 hours (44.9 as opposed to 36.3). This may have been due to the fact that at 24 hours there were two abnormally low values in the individual animal data for controls (18 and 15.5). The mean control value at 72 hours was similar to the control value at 48

hours. There was not enough information from the MLD/7 study to indicate whether or not there was any clinical toxicity at the dose levels used. Clinical observations should have been included. The study is still considered to be acceptable, however, because if the two outliers at the 24 hour point in the controls were not included, then the 24 hour control mean value would probably be consistent with the other mean control values. Also, no test dose exceeded controls and the positive controls responded appropriately.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

005316

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (84-2) - Gene Mutation in Mouse Lymphoma Cells

TOX. CHEM. NO.: 725C

ACCESSION NUMBER: 073981

TEST MATERIAL: (RS)-alpha-cyano-3-phenoxybenzyl(1RS)-cis-3(Z-2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethylcyclopropane carboxylate

SYNONYMS: PP321, Karate (formulation); constituent of cyhalothrin, PP563

STUDY NUMBER(S): VV0044

REPORT NUMBER: CTL/P/1340

SPONSOR: ICI PLC, Plant Protection Division, Fernhurst, UK

TESTING FACILITY: ICI PLC, Cntrl. Tox. Lab, Alderly Park, Macclestield, UK

TITLE OF REPORT: PP321: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells

AUTHOR(S): M Cross

REPORT ISSUED: 8/9/85

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 18c

CONCLUSION: PP321 was not mutagenic in mouse lymphoma cells under the conditions of the study up to levels of precipitation.

Classification: Acceptable

MATERIALS AND METHODS:

Chemical:

PP321 was supplied by ICI PLC, Plant Protection Division, Fernhurst, UK. Its purity was 96.6% w/w and the reference numbers were: CTL - YU2537/001/U14 Divisional Reference - SC8/85 code # BX P13. It was a cream colored solid.

Protocol:

Exponentially growing L5178Y mouse lymphoma cells were treated with the test substance for 2 hours, either with or without the presence of metabolic activation (S-9 mix from livers of AROCLOR 1254-induced Sprague Dawley rats). The test substance was removed, the cells were washed and a sample was diluted to determine survival immediately after treatment. The remaining cells were cultured for 48 hours and then grown in selective medium (trifluorothymidine, TFT) and nonselective medium (times not given) to determine the mutation frequency per viable cell. The positive

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control substances used were benzo(a)pyrene (requiring metabolic activation) and ethylmethanesulphonate (not requiring metabolic activation). The vehicle control was dimethylsulphoxide (DMSO). The test compound was considered to be a positive mutagen if the following conditions were satisfied:

The negative control data were within the normal range of the spontaneous mutation frequency for a selective agent.

The positive control showed the expected response.

The plating efficiency for the solvent control was > 50%.

A statistically significant dose related increase in mutation frequency was observed over some of the concentration range.

A dose related decrease in numbers of negative mutant wells was observed over some of the concentration range.

If at the highest dose level there was a reproducible statistically significant increase in mutation frequency above the negative control level, accompanied by > 20% survival, the test compound was also considered mutagenic provided the first 3 criteria above were also satisfied.

RESULTS:

There was little difference in survival rate with or without metabolic activation. PP321 was tested at dose levels ranging from 125 micrograms/ml to 4000 micrograms/ml and there was very little evidence of cytotoxicity at these levels. The minimum survival rate was 31%, which was observed in the first experiment at the 2000 micrograms/ml concentration. The chemical precipitated at all dose levels, especially at the higher dose levels (1000, 2000 and 4000 micrograms/ml) at which it was noted as an "agglutinous precipitation". In experiment 1, PP321 gave no mutagenic response either with or without the S-9 mix. In experiment 2, in the absence of S-9 mix, an increase of 2.9 times the spontaneous rate was observed at the 2000 micrograms/ml level. However, this concentration was in excess of the limit of solubility, as noted by marked precipitation of the test substance in the culture medium. No significant increases over solvent controls were noted for any of the other dose levels. In the third experiment, for both with and without S-9 mix, no significant increase in the mutation frequency was observed with the test material except at dose levels beyond the solubility limit [2000 and 4000 micrograms/ml (3 and 7 times background level without S-9 mix) and 1000 and 2000 micrograms/ml (with S-9 mix)]. In all experiments, the positive controls responded as expected (5 to 9 times the solvent control frequency, except in the case of the third experiment, in which the level was close to the solvent control value).

DISCUSSION:

The apparent increase in mutagenic activity both with and without metabolic activation occurred at levels where the concentration of the test chemical exceeded the solubility limit. The data at the highest dose levels should not be considered. Therefore, the test compound does not appear to be mutagenic under the conditions of the study.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

005316

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity (84-2) - In Vitro Cytogenetics TOX. CHEM. NO.: 725C

ACCESSION NUMBER: 073981

TEST MATERIAL: (RS)-alpha-cyano-3-phenoxybenzyl(1RS)-cis-3(Z-2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethylcyclopropane carboxylate

SYNONYMS: PP321, Karate (formulation), constituent of cyhalothrin

STUDY NUMBER(S): SV0200/SV0201

REPORT NUMBER: CTL/P/1333

SPONSOR: ICI PLC, Plant Protection Division, Fernhurst, UK

TESTING FACILITY: ICI PLC, Cntrl. Tox. Lab., Alderly Park, Macclesfield, Cheshire UK

TITLE OF REPORT: PP321: A Cytogenetic Study in Human Lymphocytes In Vitro

AUTHOR(S): Sheldon T, Howard CA, Richardson CR

REPORT ISSUED: July 3, 1985

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Ref. 19c

CONCLUSION: Under the conditions of bioassay, PP321 was not a clastogen.

Classification: Acceptable

MATERIALS AND METHODS:

Chemical:

The test sample of PP321 was supplied by ICI PLC, Plant Protection Division, Fernhurst, UK. The reference numbers were: CTL - Y02537/001/012, Division # SC 9/85, Batch Bx P13.

Protocol:

Blood samples were obtained from 2 healthy donors, 1 male and 1 female. Forty-eight cultures from each donor sample were initiated and maintained at 37°C in culture medium supplemented with fetal calf serum, phytohemagglutinin, penicillin and streptomycin. Forty-four hours after culture initiation, PP321 in DMSO was administered to duplicate cultures from each donor at 10 concentrations ranging from 1-1000 micrograms/ml (final dose levels chosen for the main study were 100, 500 and 1000 micrograms/ml). The dose levels were based on the limit of solubility of the test sample in DMSO at room temperature. A solvent control, DMSO and a positive

control, mitomycin C were also included. The exposure time was 4 hours (hours 44-48) at 37°C. PP321 was also tested for clastogenic activity in cultures incubated with auxiliary metabolic enzymes. Thirty minutes prior to dosing, the test sample at dose levels ranging from 1-1000 micrograms/ml in 10 microliters DMSO was pre-incubated at 37°C for 30 minutes (no explanation for this choice of incubation time) with the auxiliary metabolic enzymes (Aroclor 1254-induced rat liver S-9 and co-factor mix). At the end of the pre-incubation period, the blood cultures were added to the pre-incubated mixtures and maintained at 37°C for a further 3 hours (hours 44-47). The solvent control for this group was DMSO and the positive control was cyclophosphamide. For both sets of cultures (with and without metabolic activation) the growth medium was removed by centrifugation at the end of the exposure period and replaced by fresh growth medium. The cultures were maintained at 37°C for the remainder of the 72-hour growth period. Two hours prior to harvesting the cultures at 72 hours, the samples were treated with colchicine. Slides were prepared from the cultures and the mitotic indices were determined. One hundred cells in metaphase were analyzed from each blood culture (2/dose level, 1 for positive controls) for the incidence of chromosomal damage. For the positive controls, only those numbers of cells sufficient to confirm a positive response were analyzed.

RESULTS:

One thousand micrograms/ml PP321 was the highest dose tested, based upon its solubility in 10 microliters DMSO. For the cultures tested without metabolic activation, for donor 1, the test sample of PP321 inhibited mitosis by 21-42% and for donor 2 the sample inhibited mitosis by 0-6% over the range of dosages tested. For the cultures tested with metabolic activation, the test sample inhibited mitosis by 22-52% for donor 1 and by 17-46% for donor 2. No significant increases in aberrations over solvent controls were observed at any dose level of PP321 in cells from either donor with and without metabolic activation. The test system was shown to be sensitive as demonstrated by the response obtained by the 2 positive controls, cyclophosphamide (45-125-fold over controls) and mitomycin C (72-fold over controls).

DISCUSSION:

The main problem with this study is that only one exposure time and only one harvesting time were done. As a result, some possible aberrations may have been missed. It would have been better if multiple harvest times had been conducted, so that the first daughter cells after treatment were certain to be sampled.

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condary Reviewer: Edwin Budd
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005316

DATA EVALUATION REPORT

STUDY TYPE: Subchronic feeding 82-1 (rat) TOX. CHEM. NO.: 725C

ACCESSION NUMBER: 073980

TEST MATERIAL: (RS)-alpha-cyano-3-phenoxybenzyl, (1RS), cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate

SYNONYMS: PP321, Karate (substituent of Cyhalothrin, Grenade)

STUDY NUMBER(S): PRO584

REPORT NUMBER: CTL/P/1045

SPONSOR: ICI PLC, Plant Protection Division, Jealott's Hill, Bracknell, UK

TESTING FACILITY: ICI PLC Cntrl. Tox. Lab, Alderly Park, Macclefield, UK

TITLE OF REPORT: PP321: 90 Day Feeding Study in Rats

AUTHOR(S): Hart D, Banham PB, Chart IS, Evans DP, Gore CW, Stonard MD, Moreland S, Godley MJ, Robinson M.

REPORT ISSUED: 2/14/85

IDENTIFYING VOLUME: Volume II, Book 1, Section C, Tab 3C

CONCLUSION: The NOEL is 50 ppm and the LOEL is 250 ppm based on reduction in in body weight gain.

Classification: Core Guideline

MATERIALS AND METHODS:

Chemical:

PP321 was supplied by ICI PLC, Plant Protection Division, Bracknell, Berkshire, UK. The purity was 96.5% w/w and the reference #'s were: CTL ref. # YO2537/001/005 and batch P13. The chemical was supplied as a buff solid.

Animals:

One hundred male and female SPF Alk/AP Wistar-derived rats were obtained from the Animal Breeding Unit at ICI PLC, Alderly Park. All rats were approximately 21 days old when transported. Twenty rats per sex were assigned to each dose level: 0, 10, 50 and 250 ppm.

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Protocol:

All animals were maintained on the appropriate experimental diet for 90 days. Animals were examined pre-experimentally and once daily for abnormalities in clinical condition and/or behavior. Detailed clinical observations were conducted when the animals were weighed. Bodyweights were recorded immediately before the start of the experiment and once weekly thereafter. Food consumption was also recorded once weekly. Ten animals of each sex per group were selected for blood sampling. Samples were taken from the tail vein prior to the start of the experiment and at 4 weeks into the experiment. Blood was removed from these same animals at termination via cardiac puncture. The following hematological measurements were taken:

hemoglobin, hematocrit, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total white cell count, platelet count, differential white cell count and kaolin-cephalin and prothrombin times (at termination only).

Clinical chemistry measurements were taken on ten other male and female animals per group at the same predesignated times as the hematological measurements. The following measurements were taken:

plasma alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), triglycerides, plasma cholesterol, urea, glucose, albumin and total protein.

Urine samples were taken from the same animals from which the clinical chemistry measurements were taken, at the same predesignated times. The samples were collected over an 18 hour period. The following measurements were taken: volume, pH, specific gravity, protein, glucose, ketones and urobilinogen. During the week prior to termination, the eyes of the animals from the control and 250 ppm groups were examined using a Fison's binocular indirect ophthalmoscope.

At termination, all animals were given a full post mortem examination. The following organs were weighed: gonads (combined), spleen, adrenals (combined), kidneys (combined), liver, heart, lungs (combined with trachea attached) and brain. Tissues from the following list were removed from the high dose animals and controls and examined histopathologically together with liver, kidneys, lungs and any abnormal tissues from the lower dose groups:

adrenal glands, aorta, bladder, bone (left femur) including knee joint, brain, cecum, cervical lymph node, cervix, colon, duodenum, epididymis (L+R), eyes (+ Harderian gland), heart, jejunum, ileum, kidneys, lung, liver, mammary gland (female only (x2 inguinal)), mesenteric lymph nodes, esophagus, ovaries, pancreas, pituitary gland, prostate, rectum, salivary glands, sciatic nerves (L+R), seminal vesicles, skin (r. flank), spinal cord, spleen, sternum with bone marrow, stomach, testis (L+R), thymus, thyroid, parathyroid, trachea, uterus, voluntary muscle and abnormal tissue.

All other tissues were fixed and kept for future reference.

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Hepatic aminopyrine-N-demethylase activity (APDM) was determined from the livers of 6 male and 6 female predesignated animals. Statistical analyses were conducted on cumulative bodyweight gains, weekly and total food consumption, food utilization, biochemical and hematological data, organ weights, APDM activity and other appropriate measurements.

RESULTS:

All rats survived the study and no treatment related clinical observations were noted during the study. Body weight gain was significantly reduced for both sexes at the highest dose level. It was also reduced for the 10 and 50 ppm males for week 1. In females, bodyweight gain was slightly reduced for the lowest dose group but not for the mid-dose group. Food consumption was reduced in both sexes at the highest dose level throughout the study and in males at 50 ppm during week 1. In females, there was a slight reduction in food consumption for the 10 ppm group. There was also a small statistically significant reduction in the efficiency of food utilization for females at the 250 ppm level for weeks 1 to 4 and for overall.

Sporadic significant differences in hematologic values of treated versus control groups were noted, but were not considered to be of biological significance. ALT activity was significantly reduced for the 250 ppm males after 4 weeks. ALP activity was also significantly reduced for the 250 ppm females after 13 weeks. Plasma triglycerides were reduced for the 50 and 250 ppm males after 4 and 13 weeks, but was statistically significant only for the 250 ppm males. A small, but significant decrease in urine volume was observed at 4 weeks in the 250 ppm males.

No treatment related changes were noted in the ophthalmologic examinations. A significant increase in liver weights was observed for both sexes fed 250 ppm and for males fed 50 ppm. Ovary weights were higher for all treated groups, but significant only at the 250 ppm level. However, all values were within the historical control range.

The activity of hepatic APDM was significantly increased in both sexes fed 250 ppm and in males fed 50 ppm. No treatment related macroscopic or microscopic changes were noted at termination of the study.

DISCUSSION:

This was a well conducted study. There was a significant reduction in body weight gain and in food consumption at the highest dose level (250 ppm). The authors suggested that since in males the bodyweight gain continued to diverge from that of the controls, this was a continuing toxic effect rather than a palatability problem. In actuality, the data are borderline. The reduction in food consumption and bodyweight gain at the highest dose level could be due to lack of palatability of the diet. The reduction in ALT and ALP activities and triglyceride levels support the possibility of slight starvation of the animals. The authors also stated that the increase in liver weights and in APDM activities were indicative of an adaptive response. This is likely to be the case. Other similar insecticides have been known to induce liver

enlargement as an adaptive response (e.g. pyrethrum (85 mg/kg/day in the rat)). These results were supported by the results of the subchronic study conducted on rats with cyhalothrin, of which PP321 is the resolved enantiomer pair. In that study, the increase in APDM was also considered to be adaptive. The NOEL for this study is considered to be 50 ppm, which is also the NOEL for the cyhalothrin subchronic study in rats.

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Secondary Reviewer: Edwin Budd
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005316

DATA EVALUATION REPORT

STUDY TYPE: Subchronic oral (82-1) - rat TOX. CHEM. NO.: 271F

ACCESSION NUMBER: 073980

TEST MATERIAL: [(RS)alpha cyano-3-phenoxybenzyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethylcyclopropane-carboxylate]

SYNONYMS: Cyhalothrin, PP563

STUDY NUMBER(S): PR0418

REPORT NUMBER: CTL/P/668

SPONSOR: ICI

TESTING FACILITY: ICI PLC Cntrl. Tox. Lab, Alderly Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: Cyhalothrin Induced Liver Changes: Reversibility Study in Male Rats

AUTHOR(S): Lindsay S, Doe JE, Godley MJ, Hall M, Pratt I, Robinson M, Stonard MD

REPORT ISSUED: 2/3/82

IDENTIFYING VOLUME: Volume II, Book 1, Section C, Tab 11C

CONCLUSION: Administration of 250 ppm cyhalothrin in the diet for 28 days induced SER proliferation and an increase in APDM activity, which was reversible after 7 days. It also caused a reduction in bodyweight gain which was still apparent after a 28 day recovery period.

Classification: Acceptable for the purposes for which it was conducted but is Core Supplementary as a subchronic study.

MATERIALS AND METHODS:

Chemical:

Cyhalothrin was supplied by ICI PLC, Pharmaceuticals Division; CTL Reference # YU0102/010/005, batch ADM/46156/80. The test material was a viscous dark brown liquid, total pyrethroid content of 91.3% w/w of which 97.7% w/w was cyhalothrin.

Animals:

Twenty-one day old male Wistar-derived rats of the Alderly Park strain were supplied by the Animal Breeding Unit, ICI PLC, Alderly Park, Cheshire, UK. They were acclimatized for approximately 14 days under specific pathogen-free conditions.

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Protocol:

Two groups of 32 rats were used in the study, 1 control group and 1 group fed a diet containing 250 ppm cyhalothrin. Four sacrifice dates were scheduled at which 8 animals from each group were sacrificed: day 28 of treatment and 7, 14 and 28 days after the end of the treatment. All rats were examined once daily for abnormalities in clinical condition and behavior and once weekly for a detailed clinical examination. Bodyweights were recorded at the onset of the experiment and once weekly thereafter until termination. At termination, all the liver weights were recorded. The livers were also examined by electron microscopy and stored in formol saline. Liver sections from each rat were also taken for measurement of hepatic aminopyrine-N-demethylase activity (APDM). Statistical analyses were conducted where appropriate.

RESULTS:

The diets containing cyhalothrin were within 6% of the nominal concentrations. All rats remained in good clinical condition throughout the study. A statistically significant reduction in bodyweight gain was observed for all the groups except the 28-day recovery period group. After adjustment for bodyweight, the liver weights of the treated groups were similar to the control groups. SER proliferation was significantly higher in the treated group than in the control group, however, the animals showed complete recovery by day 7 post treatment. No macroscopic changes were evident at post mortem, therefore, no histopathology was performed. A statistically significant increase in APDM activity was noted at the end of the 28-day treatment period, which was also back to normal by day 7 post-treatment.

DISCUSSION:

This study was not meant to be a subchronic study per se. The data indicated that the effects on the liver, i.e. SER proliferation and increase in ADPM activity were reversible after a short recovery period. This was considered to be a physiological adaptive response rather than a toxic response. This is likely to be the case because the effects were clearly reversible after a short recovery period. The reduction in bodyweight gain persisted up to the 28 day recovery period (although not statistically significant at this point). This was considered to be due to the toxic action of the chemical and is supported by data from other subchronic oral studies conducted on this chemical at similar levels. This study is acceptable for the purpose for which it was performed, but can only be considered to be Core Supplementary as a subchronic study for this compound.

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005316

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (85-1) - rat TOX. CHEM. NO.: 271F

ACCESSION NUMBER: 073981

TEST MATERIAL: (R,S) alpha-cyano-3-phenoxybenzyl (1R,S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate and the radiolabelled ^{14}C version

SYNONYMS: Cyhalothrin, PP563, (Grenade is the formulation)

STUDY NUMBER(S): URU169

REPORT NUMBER: CTL/P/1014

SPONSOR: ICI PLC Plant Protection Division, Bracknell, Berks, UK

TESTING FACILITY: ICI PLC Cntrl. Tox. Lab, Alderly Park, Macclesfield, UK

TITLE OF REPORT: Cyhalothrin: Bioaccumulation in the Rat

AUTHOR(S): Prout MS

REPORT ISSUED: July 31, 1984

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 26C

CONCLUSION: Cyhalothrin is taken up slowly by fat and released slowly. It is rapidly released by the blood, kidneys and liver. The data indicate that the rate of metabolism of both enantiomer pairs of cyhalothrin is likely to be identical, which means that the rate of metabolism of PP321 is likely to be identical to cyhalothrin.

Classification: This study, in combination with previous metabolism and distribution studies conducted on cyhalothrin (previous submission), is classified as Core Guideline.

MATERIALS AND METHODS:

Chemical:

Cyhalothrin and ^{14}C-cyhalothrin were obtained from ICI PLC, Plant Protection Division, Bracknell, Berks, UK. The purity of the non-radiolabelled chemical was 92.2%. The radiochemical purity of the radiolabelled chemical was 98.6%. The position of the radiolabel was on the cyclopropane ring.

Animals:

Adult male Alpk/AP strain rats were obtained from the Alderly Park animal breeding unit.

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Protocol:

The dosing solution was prepared by mixing unlabelled cyhalothrin, radiolabelled cyhalothrin and corn oil to achieve a final concentration of 0.5 mg/ml cyhalothrin in corn oil (approximately 0.05 MBq/mg cyhalothrin). Animals were dosed once daily for up to 119 consecutive days (bodyweight dependent doses; controls received corn oil alone). Groups of 3 treated and 1 control were sacrificed after every 7 doses (24 hours following last dose), for up to 77 dosages and then after 91, 105 and 119 doses. Upon sacrifice, samples of blood and fat and the liver and kidneys were taken for radioactivity analysis. The liver and kidneys were weighed. The samples were combusted in a Packard Tricarb model B306 sample oxidizer and analyzed for radioactivity. Oxidation efficiencies of <92% were rejected. In addition, fat samples were extracted with hexane and dimethyl formamide, separated by HPLC and counted for radioactivity.

RESULTS:

During the dosing period, the levels of radioactivity in the blood remained between 0.10 and 0.59 micrograms/g blood, average peaking at 0.2 micrograms/g blood. After an initial rise, the levels of radioactivity in liver and kidney appeared to plateau at 2.5 and 1.2 micrograms/g respectively after 70 days of dosing. The levels of radioactivity in these three tissues declined rapidly upon cessation of dosing (levels in kidney and blood barely detectable after 5 weeks and levels in liver declining rapidly at first and then elimination parallelling that of fat). Levels in fat increased with time to a level of approximately 9 micrograms/g at 119 days. Upon cessation of dosing, these levels declined by a first order process (typical exponential decline with time). Separation of the fat extracts by HPLC gave 2 main peaks, corresponding to the 2 enantiomer pairs of cyhalothrin. The ratio of the pairs present in fat was the same as in the dosing solutions. The half-life of cyhalothrin in fat was calculated to be 30.5 days.

DISCUSSION:

Cyhalothrin was taken up slowly in the fat and released slowly. This was not the case in the other tissues. It was eliminated fairly rapidly. In the case of the liver, the small amounts remaining were probably due to amounts being slowly released from the fat tissue. In addition, the data indicated that the rates of metabolism are likely to be identical for both enantiomeric pairs since their ratio was the same for both the dosing solution and the amounts found in fat. Therefore, the rate of metabolism of PP321, which is one of the two pairs of enantiomers, is likely to be identical to the rate of metabolism of cyhalothrin (PP563).

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005316

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (85-1) - rat

TOX. CHEM. NO.: 271F
725C
725B

ACCESSION NUMBER: 073981

TEST MATERIALS: (R,S) alpha-cyano-3-phenoxybenzyl (1R,S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate; (R+S) alpha-cyano-3-phenoxybenzyl(1S+R)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate; and (R+S) alpha-cyano-3-phenoxybenzyl (1R+S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate

SYNONYMS: Cyhalothrin, PP563; PP321; R157836 respectively

STUDY NUMBER(S): UR0178

REPORT NUMBER: CTL/P/1214

SPONSOR: ICI PLC, Plant Protection Division

TESTING FACILITY: ICI PLC, Cntrl. Tox. Lab., Alderly Park, Macclesfield, UK

TITLE OF REPORT: PP321: Comparative Absorption Study in the Rat (1 mg/kg)

AUTHOR(S): Prout MS and Howard EF

REPORT ISSUED: March 19, 1985

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 27C

CONCLUSION: The results indicate that the absorption, distribution, metabolism and excretion patterns of PP321 and cyhalothrin following a single 1 mg/kg dose in the male rat are identical.

Classification: When taken with previously submitted metabolism studies, this study is Core Guideline.

MATERIALS AND METHODS:

Chemical:

All chemicals were obtained from ICI PLC Plant Protection Division.
R157836 was prepared from cyhalothrin by HPLC.
Cyhalothrin: Unlabelled purity 97.4% w/w, CTL ref. # Y00102/034/001. Labelled chemical prepared by mixing equal proportions of the ^{14}C-PP321 and ^{14}C-R157836.
PP321: Unlabelled purity 99.0% w/w, CTL ref. # YO2537/045/001. Labelled chemical labelled in cyclopropane ring, specific activity 1.95GBq/mmole and radiochemical purity of >98%. CTL ref. # YO2537/044/001.
R157836: Unlabeled purity 93.5%, CTL ref. # YO4369/002/001. Labelled chemical labelled in cyclopropane ring, specific activity 1.97GBq/mmole and radiochemical purity of >98%. CTL ref. # YU4369/044/001.

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Animals:

Twelve male Alpk/AP rats (170-250g) were obtained from the Alderly Park Animal Breeding Unit. They were kept in individual metabolism cages.

Protocol:

Three dosing solutions were prepared such that a dose level of 4 ml/kg bodyweight was equivalent to a nominal dose level of:

Dose 1: 1 mg/kg PP321 + 1MBq/kg ^{14}C-PP321
Dose 2: 1 mg/kg PP321 + 1MBq/kg ^{14}C-PP321 + 1 mg/kg R157836
Dose 3: 1 mg/kg cyhalothrin + 1 MBq/kg ^{14}C-cyhalothrin

Four animals per dose group were given one oral dose (4 ml/kg) of the selected dose. Urine and feces were collected over dry ice at 24 hour periods for 3 days and retained at -20°C for analysis. Cage washings were also retained. Upon sacrifice, samples of blood and fat, and the liver and kidneys were removed and retained at -20°C for analysis. The livers and kidneys were homogenized in water and the fat samples were homogenized without water. Feces were homogenized in methanol. Samples of blood, liver, kidneys, fat and fecal residue were combusted and analyzed for radioactivity. Oxidation efficiencies of <92% were rejected. Samples of urine and cagewash were diluted and counted directly.

Zero to 24 hour urine samples and the 0-24 hour and 24-48 hour samples of fecal extracts were retained from each animal and analyzed on thin layer chromatography (TLC) in the following solvent systems:
chloroform : methanol : acetic acid 18:1:1
butan-1-ol : acetic acid : water 9:2:1
The precise location of radiolabel was confirmed by autoradiography.
Two tailed Student's t-tests were used to compare one group with another group.

RESULTS:

The total excretion of radioactivity from the 3 groups was very similar. There were no statistically significant differences in the total urinary or the total fecal excretion of radioactivity between the 3 groups. The authors stated that the very low levels of radioactivity found in the blood were close to the limit of detection, thus the apparent differences in levels of ^{14}C-PP321 and ^{14}C-cyhalothrin was probably spurious. They also stated that the differences in liver concentrations between dose groups II and III disappear when the comparison is made on the basis of percentage dose left in the liver at termination. The mean concentrations of radioactivity in the fat of rats dosed with either ^{14}C-PP321 or ^{14}C-cyhalothrin were nearly identical (0.25 and 0.26 microgram equivalents/g fat respectively. In addition, the residue level of radioactivity in fat of animals in dose group II was not significantly different from either of the other groups.

The methanol extract of the 0-48 hour feces from rats in the 3 groups contained a mean of 65% of the total material excreted via the feces in this period. More than half of the material was unchanged cyhalothrin and the major metabolites present were common to all groups. The methanolic trituration of freeze dried urine (0-24 hours) extracted a mean of 90% of the radioactivity present in the urine from rats in all groups. According to the authors, the major peak of radioactivity when chromatographed by TLC was probably the glucuronide of cyhalothrin acid. No unchanged PP321 or cyhalothrin was excreted in the urine, however, the free cyhalothrin acid was a significant urinary metabolite in all groups accounting for between 3-9% of the material present in the day 1 urine.

DISCUSSION:

The results of this study indicate that the absorption, metabolism, excretion and tissue distribution of ^{14}C-PP321 and ^{14}-cyhalothrin are indistinguishable from one another. These results are compatable with previous studies.

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005316

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity (83-2) - mouse TOX. CHEM. NO.: 271F

ACCESSION NUMBER: 073981

TEST MATERIAL: (RS) alpha-cyano-3-phenoxybenzyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate

SYNONYMS: Cyhalothrin, (Grenade is formulation name), PP563

STUDY NUMBER(S): CTL/C/1260

REPORT NUMBER: ICI 395/83668

SPONSOR: ICI PLC, Cntrl. Tox. Lab, Alderly Park, Macclesfield, UK

TESTING FACILITY: Huntingdon Research Cntr. Huntingdon, Cambridgeshire, UK

TITLE OF REPORT: Cyhalothrin. Potential Tumorigenic and Toxic Effects in Prolonged Dietary Administration to Mice (Addendum to Final Report)

AUTHOR(S): Colley J, Dawe S, Heywood R, Almond R, Gibson WA, Gregson R, Chirukandath G

REPORT ISSUED: July 26, 1984

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 2$C

CONCLUSION: Cyhalothrin was not oncogenic to mice under the conditions of the study (see conclusion in original DER). The addendum does not change the original review.

Classification: Core Minimum

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005316

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral (82-1) rat TOX. CHEM. NO.: 271F

ACCESSION NUMBER: 073980

TEST MATERIAL: (RS)alpha-cyano-3-phenoxybenzyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate and (RS)alpha-cyano-3-phenoxybenzyl (EZ)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate

SYNONYMS: Cyhalothrin, PP563 (active ingredient of Grenade) for first test chemical and PP564 for second test chemical

STUDY NUMBER(S): PRO337

REPORT NUMBER: CTL/P/1056

SPONSOR: ICI PLC Plant Protection Division, UK

TESTING FACILITY: ICI PLC, Cntrl. Tox. Lab., Alderly Park, Macclesfield, UK

TITLE OF REPORT: PP563: 28-Day Feeding Study in Rats - Summary Report

AUTHOR(S): Tinston DJ, Banham PB, Chart IS, Gore CW, Pratt I, Scales MDC, Weight TM.

REPORT ISSUED: 7/12/84

IDENTIFYING VOLUME: Volume II, Book 1 of 2, Section C, Tab Ref. 9C

CONCLUSION: For male rats effects were noted at the lowest dose level PP563, 20ppm. For females, the NOEL was 20 ppm. PP564 was less toxic than PP563, indicating that the cis isomer is more toxic than the trans isomer.

Classification: Not Core Guideline, but acceptable for the purposes for which it was performed.

MATERIALS AND METHODS:

Chemical:

PP563 was given the following references: CTL - Y00102/006/001 and Plant Protection Batch P5. It had a purity of 89.0% w/w (100% cis isomer). PP564 was given the following references: CTL - Y00102/001/001 and Plant Protection Batch P5. It had a purity of 84.0% w/w (50:50 cis:trans isomers). Both were viscous, pale yellow liquids.

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Animals:

Male and female Alpk/AP (Wistar-derived) rats were obtained from the Animal Breeding Unit at ICI PLC, Alderly Park, Macclesfield, Cheshire, UK. The rats were 3 weeks old and were acclimated for one week. The animals were supplied in two groups, one group arriving a week ahead of the other group.

Protocol:

Six groups of 16 male and 16 female rats were fed the experimental diets at the following dose levels for 28 days: 0, 20, 100, 250, 500, and 750 ppm (PP563); and 500 and 750 ppm (PP564). All rats were observed once daily throughout the experimental period for any clinical signs of toxicity. The eyes of all rats from the control, 500 and 750 ppm groups (PP563) were examined pre-experimentally and during the week prior to termination with an ophthalmoscope with and without a mydriate. Bodyweights were recorded weekly and food consumption was recorded daily for the first week and weekly thereafter.

Clinical Chemistry:

The following clinical chemistry parameters were measured in up to 8 designated male and female rats per group prior to the experimental phase and at termination: plasma urea, glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and triglyceride and plasma cholesterol levels (at termination only).

Urinalysis:

Urinalysis measurements were taken from up to 4 male and 4 female rats prior to the experimental phase and at termination. The rats were given an oral water load at 2.5 ml/100g bodyweight and the urinary volume, pH, specific gravity and urinary sediments were measured. The animals were then deprived of water for 18 hours during which time the urine was collected for analysis of urinary volume, pH, specific gravity, protein, glucose, bilirubin and ketones.

Hematology:

The following hematological measurements were taken pre-experimentally and terminally from up to 8 male and 8 female animals per group: hemoglobin, total white cell count, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, hematocrit, differential white cell count and platelet count. The morphological appearance of the red cells were also examined. At termination, in addition to the above, prothrombin and kaolin/cephalin time tests were conducted and 2 bone marrow smears from the right femurs of all rats were examined for any cytological abnormalities.

Pathology:

Any rats found dead or moribund during the study received a full post mortem examination and tissues were submitted for histopathological examination. The weights of the following organs were recorded from up to 8 male and females rats per group: gonads (combined), spleen, adrenals (combined), kidneys (combined), liver, thymus, heart, lungs (combined), brain and pituitary. The livers from these animals (except the PP564 livers) were fixed in formol corrosive for histopathological examination. The livers from the PP564 group along with the following tissues from 8 male and 8 female animals per group were fixed in formol saline: salivary glands (parotid, sub-maxillary and sub-lingual), cervical lymph node, mammary tissue, voluntary muscle, testes, epididymides, protate and seminal vesicles or ovaries, uterus and cervix, urinary bladder, spleen, pancreas, stomach, duodenum, jejunum, ileum, mesenteric lymph node, caecum, colon, adrenals, kidneys, liver, thyroid, aorta, trachea, esophagus, thymus, heart, lungs, eyes, sciatic nerves, brain and spinal cord. The left sciatic and posterior tibial nerves from 4 male and 4 female controls and 750 ppm PP563 groups were fixed in formol glutaraldehyde and examined. All remaining animals received a gross post mortem examination and only abnormal appearing tissues were submitted for histopathological examination. Livers from a designated 6 male and 6 female animals from all groups were taken for measurement of hepatic aminopyrine-N-demethylase activity. These livers were the same as those taken for measurement of weight and examination by electron microscope. For the electron microscopy, samples were taken from the median lobes from the preselected male and female animals from control, 20, 100 and 250 ppm groups (PP563). Smooth endoplasmic reticulum (SER) was quantified using the point counting method of Weibel.

RESULTS:

Dietary Concentrations:

Concentrations of PP563 and PP564 were within 10% of the nominal values except for the 500 ppm PP563 and 500 ppm PP564 diets where the mean concentrations were 83% and 89% respectively. PP563 was shown to be stable in the diet for up to 30 days after preparation.

Mortalities:

Three male and three female rats receiving 750 ppm PP563 in the diet were found to be either dead or moribund. As a result, a second batch of animals already scheduled to start one week later were fed 500 ppm instead (this also included a second batch of PP564 animals). At 750 ppm, 2 more female rats died, one after 14 days and one after 27 days. No other deaths occurred during the study.

Clinical Observations:

Clinical observations included high-stepping gait, severe ataxia, hypersensitivity to external stimuli, piloerection and excessive salivation at the 750 and 500 (less severe) ppm (PP563) levels and similar but transient effects at the 250 ppm level. At 100 ppm, one male showed high stepping gait on day 3. Also at the lower levels there was occasional evidence of slight hypersensitivity to external stimuli. The clinical effects observed with PP564 were comparable but less severe: the effects noted at the 750 ppm level were similar to those noted at the 500 ppm level of PP563 and the effects observed at the 500 ppm level were similar to those noted at the 250 ppm level of PP563.

Bodyweight Gain and Food Consumption:

Statistically significant decreases in bodyweight gain were noted for male and female groups receiving either PP563 or PP564 at dietary concentrations of 250 ppm or greater (except for bodyweight gains of males receiving 500 ppm PP564). Statistically significant reductions in food consumption were also observed in both male and female rats fed levels of 250 ppm or greater for both PP563 and PP564.

Clinical Chemistry and Urinalysis:

Reductions in plasma triglyceride levels were noted in males receiving either 500 or 700 ppm PP563 and to a lesser extent in females receiving 750 ppm PP563 and males receiving either 500 or 750 ppm PP564. Dose-related decreases in protein excretion levels in the urine were observed in males receiving either 500 or 750 ppm PP563.

Organ Weights:

Statistically significant increases in liver weights (after adjustments for bodyweights) were observed in the 250 and 500 ppm dose groups (PP563) and in the 750 ppm (PP564) dose group. At 750 ppm 563, the large bodyweight reduction distorted the organ weight analysis. There was some evidence of increased testes weights and decreased ovary weights at the 500 and 750 ppm levels of PP563. There was a dose-related reduction in the heart weight of males fed diets containing PP563 which was statistically significant down to 250 ppm. There was also some evidence for reduction in spleen, brain and thymus weights in groups which grew less than controls.

Histopathology:

Male and female rats dying or killed in extremis showed thymic atrophy, and enlargement, vacuolation and differential staining of the cortical cells of the adrenals. In males, incomplete spermatogenesis and reduction of seminal vesicular secretion was evident. No changes in the nervous system were present. No other changes were noted.

Hepatic Aminopyrine Demethylase Activity:

A dose-related increase in APDM activity was observed in male rats receiving 20 ppm and above (PP563), in females receiving 100 ppm and above (PP563) and in PP564 but to a lesser extent.

Electron Microscopy:

There was a statistically significant increase in SER proliferation (greater in males than in females) which did not show any dose-response effect. The effect was observed in males at dose levels of 20, 100 and 250 ppm PP563 and in females at 250 ppm PP563. One female rat receiving 250 ppm PP563 showed marked vacuolation of hepatocyte cytoplasm, as a consequence of dilatation of endoplasmic reticulum.

DISCUSSION:

The results of this study confirmed the results of another previously submitted 28-day study on cyhalothrin in rats (Moyes et al. 1984) conducted at dose levels of 1 - 250 ppm. Clinical observations indicated signs of neurotoxicity, characteristic of synthetic pyrethroid toxicity. Evidence of decreased bodyweight gain and food consumption was also noted, as well as increased ADPM activity and proliferation of SER. As evidenced by comparing the results from testing PP564 with the results from PP563, it appears that the cis component is the more toxic of the 2 isomers. It should be noted that even at the lethal dose of 750 ppm PP563, no histopathological changes were observed in the peripheral nerves, even when accompanied by neurotoxic signs. The liver hypertrophy accompanied by increases in liver weight, APDM activity and SER proliferation are characteristic of effects due to pyrethroid administration. These effects are considered to be adaptive in this case. The authors stated that the histopathological changes noted in the animals that died were due to stress rather than PP563 toxicity, especially since there was no sign of these changes in the animals that survived.

The purpose of the study was to find the highest dose useful for a longer term study and to compare the toxicity of PP563 with PP564. It was recommended that for longer term studies, dosages higher than 250 ppm should not be used. This study is not Core Guideline because the exposure time was only 28 days and only 8 of the animals per sex per dose group were examined for many of the measurements taken. However, the study is acceptable for the purpose that it was conducted.