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

NOV 6 1996

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Registration of Foli-R-Fos 400 (EPA Reg. No. 069579-R) containing 45.5% potassium salts (mono- and di) of Phosphorous Acid to be used as Fungicide to control Phytophtora and Pythium of Ornamentals. Review of Mutagenicity. MRID No. 439058-10; Submission No. S505790; PD Barcode D226397

FROM: Freshteh Toghrol, Ph.D., Chemist F Toghrol 10/31/96
Biopesticides & Pollution Prevention Division (7501W)

THRU: James Thomas McClintock, Ph.D., Team Leader
Biopesticides & Pollution Prevention Division (7501W) JTMc 11/6/96

TO: Rita Kumar, Regulatory Action Leader
Biopesticides & Pollution Prevention Division (7501W)

Action

U.I.M. Agrochemicals (Aust.) PTY, LTD, requests registration of the Foli-R-Fos 400 (EPA Reg. No. 069579-R) containing 45.5% potassium salts (mono- and di) of phosphorous acid as its active ingredient to be used as fungicide to control Phytophtora and Pythium of Ornamentals.

To support this registration, U.I.M. Agrochemicals (Aust.) PTY, LTD has submitted the mutagenicity data (MRID No. 439058-10) a Salmonella typhimurium reverse mutation assay (Ames)/mammalian of Foli-R-Fos 400.

Conclusions and Discussion

1. A reverse mutation assay (Ames assay was used to evaluate the product's potential to induce histidine reversion (mutagenic response) in five strains of *Salmonella typhimurium*. In a Salmonella/microsome plate incorporation assay, strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to FOLI-R-FOS 400 at concentrations of 33, 100, 333, 1000, 3333 and 10,000 μg/plate, with and without exogenous metabolic activation (S9-mix). Preparations for metabolic activation were made from Aroclor 1254 induced male Fischer 344 rat livers. The test material was delivered in ultra-pure sterile water.

   FOLI-R-FOS 400 was not cytotoxic at any experimental point in this study and no precipitation of test material was seen. In the absence of cytotoxicity, 10,000 μg per plate is an acceptable upper concentration. Positive and solvent control values were appropriate and two independent experiments were conducted. **There was no evidence of induced mutant colonies over background in any strain at any dose tested, with or without S9-mix.**

This study is classified as an acceptable study. It satisfies the guideline requirements for a gene mutation study (152-17).

cc: T. McClintock, F. Toghrol, Rita Kumar, BPPD Subject file.
F. Toghrol, CS#1: BPPD: Tel (703) 308-7014:10/31/96

EPA Reviewer: Freshteh Toghrol, Ph.D.
Biopesticides and Pollution Prevention Division

F. Toghrol Date: 10/31/96

DATA EVALUATION REPORT

STUDY TYPE: Salmonella/mammalian activation gene mutation assay (84-2(a))

PC CODE: 076416

DP BARCODE: D226397

CASE: 046750

MRID NUMBER: 43905810

TEST MATERIAL: Mono- and di-potassium salts of Phosphorous Acid

SYNONYMS: FOLI-R-FOS 400

STUDY NUMBER: IRI Report No. 10664; IRI Project No. 755665; CSI Project No. 94049.UIML

SPONSOR: U.I.M. Agrochemicals (Aust.) Pty Limited; PO Box 72, Brisbane Market, Queensland 4106, Australia, Sponsor's Representative: Compliance Services International, 1112 Alexander Avenue, Tacoma, WA 98421

TESTING FACILITY: Inveresk Research International, Tranent, EH33 2NE, Scotland

TITLE OF REPORT: Potassium salts of phosphorous acid - Testing for mutagenic activity with *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100

AUTHORS: Willington, S. E. and C. G. Riach

REPORT ISSUED: December 6, 1994 (Study completion date)

EXECUTIVE SUMMARY:

In a Salmonella/microsome plate incorporation assay (MRID 43905810), strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to FOLI-R-FOS 400 at concentrations of 33, 100, 333, 1000, 3333 and 10,000 μg/plate, with and without exogenous metabolic activation (S9-mix). Preparations for metabolic activation were made from Aroclor 1254 induced male Fischer 344 rat livers. The test material was delivered in ultra-pure sterile water.

FOLI-R-FOS 400 was not cytotoxic at any experimental point in this study and no precipitation of test material was seen. In the absence of cytotoxicity, 10,000 μg per plate is an acceptable upper concentration. Positive and solvent control values were appropriate and two independent experiments were conducted. **There was no evidence of induced mutant colonies over background in any strain at any dose tested, with or without S9-mix.**

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A. MATERIALS

1. Test material: Mono- and di-potassium salts of Phosphorous Acid

Description: clear colorless liquid
Batch No.: 22 44 3
Purity: 65.26% m/v potassium phosphite salts
Stability of compound: responsibility of sponsor
CAS No.: not provided
Structure: not provided
Solvent used: water, ultra-pure

2. Control materials

Solvent/final concentration: ultra-pure water / 0.1 ml/plate
Positive:

Non-activation:		
Sodium azide	1.0 µg/plate	TA100, TA1535
2-Nitrofluorene	1 µg/plate	TA98, TA1538
9-Aminoacridine	80 µg/plate	TA1537
Activation:		
2-Aminoanthracene	2 µg/plate	TA1535, TA1537
2-Aminoanthracene	0.5 µg/plate	TA98, TA100, TA1538

3. Activation

S9 derived from
_x_ Aroclor 1254 _x_ induced _x_ rat _x_ liver
Male Fischer 344 rats were used.

S9-mix composition:
Co-factor solution: (in 0.05 M phosphate buffer (pH 7.4))

NADP, sodium salt	4 mM
glucose-6-phosphate, sodium salt	25 mM
$MgCl_2 \cdot 6H_2O$	8 mM
KCl	33 mM

Supernatant from liver homogenate:
S9-mix was 9 parts co-factor solution plus 1 part liver homogenate

4. Test organisms

S. typhimurium strains
__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538

Properly maintained? YES
Checked for appropriate genetic marker? YES

5. Test compound concentrations used

Non-activated conditions:
Preliminary toxicity test: 33, 100, 333, 1000, 3333 and 10,000 μg/plate (TA100 only)
Mutagenicity test: 33, 100, 333, 1000, 3333 and 10,000 μg/plate

Activated conditions:
Preliminary toxicity test: 33, 100, 333, 1000, 3333 and 10,000 μg/plate (TA100 only)
Mutagenicity test: 33, 100, 333, 1000, 3333 and 10,000 μg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay

_x_ standard plate test
__ pre-incubation (__ minutes)
__ "Prival" modification
__ spot test
__ other (describe in a.)

2. Protocol

Five ml of a sterile solution of 1.0 mM L-histidine HCl / 1.0 mM biotin was added to 100 ml molten top-agar (0.6% Difco Bacto-agar, 0.6% NaCl). Two-ml aliquots of the top-agar mix were dispensed into small sterile tubes and the following added in order: 0.5 ml S9-mix or 0.05 M phosphate buffer (pH 7.4); 0.1 ml bacteria suspension (about 2 x 10^8 cells); and 100 μl of solvent or test solution. After mixing, the tube contents were poured onto minimal agar plates containing 25 ml of 1.5% BBL purified agar in Vogel-Bonner Medium E with 2% glucose. After the agar solidified, the plates (three per test point) were incubated at 37 °C for 2 days. Colonies were counted (0.1 mm or larger) using a Biotran III automated counter. Plates were also examined for precipitates and microcolony growth.

The testing laboratory's criteria for an acceptable test were:

1. The bacteria had appropriate karyotype.
2. Revertant count on at least 2 of the vehicle control plates was within 4-30 for TA1535, 1-20 for TA1537, 10-60 for TA98, 60-200 for TA100 and 5-35 for TA1538.

3. On at least 2 of the positive control plates the revertant count was ≥ 2 times the mean vehicle control count except for TA100 where the requirement was 1.5 times the control count. If the mean revertant count on vehicle control plates was less than 10, then a count of 10 was assumed for the determination.
4. No toxicity or contamination was observed in at least 4 dose levels.
5. If a mutagenic response was observed, no more than one dose level could have been discarded before the dose which gave the highest significant mean colony number.

C. REPORTED RESULTS

1. Preliminary cytotoxicity assay

Six concentrations of FOLI-R-FOS 400, ranging from 33 to 10,000 μg/plate, were tested with and without S9-mix in TA100. Results of the cytotoxicity assay are presented in Appendix Table 1 (MRID 43905810, p.26). Although the background lawn was presumably examined for thinning (to evaluate cytotoxicity as part of the testing laboratory's standard protocol), the text states only that no toxicity to the bacteria was observed and Table 1 gives only revertant counts. A decrease in the number of revertant colonies per plate with increasing concentrations, also indicative of toxicity, was not seen. In the absence of cytotoxicity or solubility limitations, an upper concentration of 10,000 μg/plate was selected for the mutagenicity tests.

2. Mutagenicity assay

There was no evidence of a mutagenic effect of FOLI-R-FOS 400 in any bacteria strain at any concentration tested in this study, with or without S9-mix in either Experiment 1 or 2. There was no observable cytotoxicity or precipitation of test material at any experimental point. The positive and solvent control values were appropriate. Results of Experiment 1, with and without S9-mix, are summarized in Appendix Tables 2 and 3 respectively (MRID 43905810, pp.27 and 28). Comparable results from Experiment 2 are summarized in Appendix Tables 4 and 5 (MRID 43905810, pp. 29 and 30).

D. REVIEWER'S DISCUSSION/CONCLUSIONS

This is an acceptable study. Although the upper dose was not limited by cytotoxicity or solubility, FOLI-R-FOS 400 was tested to a sufficiently high dose (10,000 μg/plate), exceeding the limit dose of 5000 μg/plate for this assay. Positive and negative control values were appropriate. There was no evidence in two independent experiments of revertant induction above solvent control values at any concentration tested in any strain, with or without S9-mix.

E. Was test performed under GLPs (is a quality assurance statement present)? YES

F. Appendix attached? NO CBI appendix attached

APPENDIX

MRID# 439058-10

Page_____ is not included in this copy.

Pages 8 through 12 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.

✓ 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.
