US EPA ARCHIVE DOCUMENT

7/25/96

# DATA EVALUATION REPORT

AZOXYSTROBIN

STUDY TYPE: SALMONELLA/MAMMALIAN ACTIVATION
GENE MUTATION ASSAY (84-2)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 95-19X

Primary Reviewer:
B. L. Whitfield, Ph.D.

Signature: B L Whitfield
Date: 6/1-/96

Secondary Reviewers:
Cheryl B. Bast, Ph.D., D.A.B.T.

Signature: C B Bast
Date: 6-5-96

Robert H. Ross, M.S., Group Leader

Signature: RHR
Date: 6-5-96

Quality Assurance:
Susan Chang, M.S.

Signature: S S Chang
Date: 6-5-96

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: I. Mauer, Ph.D. _____, Date 07-18-96
Toxicology Branch I (7509C)
EPA Secondary Reviewer:
M. Copley, D.V.M., D.A.B.T. _____, Date 7/25/96
Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella/mammalian activation gene mutation assay OPPTS 870.5265[1], 870.5100 [§84-2]

DP BARCODE: D218319 SUBMISSION CODE: S489692
P.C. CODE: 128810 TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): ICIA5504 (Azoxystrobin)(97.2% w/w)

SYNONYMS: E5504

CITATION: Callander, R. (1992) ICIA5504 - An evaluation of mutagenic potential using S. typhimurium and E. coli. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/P/3790, October 19, 1992. MRID 43678146. Unpublished.

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

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 43678146), strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and strains WP2P and WP2PuvrA of *Escherichia coli* were exposed to ICIA5504 (97.2% w/w) in DMSO at concentrations of 100, 200, 500, 1000, 2500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). S9-mix was prepared from phenobarbital plus β-naphthoflavone induced male Alderley Park (Alpk:APfSD) rat liver.

ICIA5504 was tested up to 5000 µg/plate, an acceptable upper concentration. Test material precipitation and thinning of the background lawn was seen at this upper concentration. Both a standard plate assay and a pre-incubation assay were used and no significant, dose-related increase in the number of revertants per plate over solvent control values was seen in any strain in the presence or absence of S9-mix. The positive and solvent controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

[1] 870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity [bacterial reverse gene mutation] data.

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

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test material: ICIA5504

Description: light brown solid
Lot/Batch #: P39/D7534/22
Purity: 97.2 w/w % a.i.
Stability of compound: responsibility of sponsor
CAS #: not provided
Structure: not provided
Solvent used: DMSO

2. Control materials

Negative:
Solvent/final concentration: DMSO/100 µl/plate
Positive:
Nonactivation:

| | | |
|---|---|---|
| Sodium azide | 0.5, 1.0, 2.0 µg/plate | TA100, TA1535 |
| ICR191 | 0.5, 1.0, 2.0 µg/plate | TA1537 |
| Daunorubicin | 0.2, 0.5, 1.0 µg/plate | TA98 |
| Mitomycin C | 0.2, 0.5, 1.0 µg/plate | WP2P |
| ENNG | 0.2, 0.5, 1.0 µg/plate | WP2PuvrA |

(1-Ethyl-3-nitro-1-nitrosoguanidine)

Activation:
2-Aminoanthracene µg/plate
0.5, 1.0, 2.0 - TA1535, TA1537
0.2, 0.5, 1.0 - TA98, TA100
5.0, 10, 20 - WP2P
1.0, 2.0, 5.0 - WP2PuvrA

3. Activation

S9 derived from
____ Aroclor 1254 ____ induced rat ____ liver
____ phenobarbital ____ non-induced ____ mouse ____ lung
____ none _x_ other

S9 from combined phenobarbital plus β-naphthoflavone induced male Alderley Park (Alpk:APfSD) rat liver was used.

Describe S9 mix composition:
Vol per 30 ml S9-mix

| | |
|---|---|
| S9 fraction | 3 ml |
| Sucrose-Tris-EDTA buffer | 7 ml |

| | |
|---|---|
| Co-factor Solution* | 20 ml |
| *$Na_2HPO_4$ | 100 mM |
| Kcl | 33 mM |
| Glucose-6-phosphate | 5mM |
| NADP (Na salt) | 4 mM |
| $MaCl_2$ | 8 mM |

4. Test organisms

*S. typhimurium* strains
_____ TA97 __x__ TA98 __x__ TA100 ____ TA102 ____ TA104
__x__ TA1535 __x__ TA1537 _____ TA1538;

list any others: *E. coli* WP2P and WP2PuvrA

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used

Nonactivated conditions:
all strains, triplicate plates
100, 200, 500, 1000, 2500, 5000 µg/plate
Activated conditions:
all strains, triplicate plates
100, 200, 500, 1000, 2500, 5000 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay

_x_ standard plate test (first assay)
_x_ pre-incubation (_60_ minutes) (second assay)

2. Protocol

Standard plate test
For each experimental point (strain, dose, with or without S9-mix), 0.1 mL aliquots of an overnight culture of bacteria were added to three sterile plastic bijou bottles (five for solvent control and 2 each for the positive controls). S9-mix or buffer (0.5 mL) was added to each bottle followed by 0.1 ml of the desired concentration of test material. Two ml of molten top-agar was then added to each bottle with sufficient force to mix the contents. The mixture was poured rapidly onto the surface of a Vogel Bonner plate and allowed to gel. The plates were labeled and incubated inverted at 37°C for 3 days in the dark.



60C

Pre-incubation test

The protocol was the same as that of the standard plate test except the volume of test material added was 0.02 mL with volume made up to 0.1 mL with phosphate buffered saline and a 60 min preincubation period at 37°C occurred prior to adding the topagar.

For both protocols, following 3 days of incubation, the plates were examined for the presence of a background lawn, lack of contamination and for proper positive control response. The number of revertant colonies was counted with an automatic colony counter. Statistical significance was determined by Student's t-test with values of $p<0.01$ being considered positive and values of $p<0.05$ possibly significant.

## II. REPORTED RESULTS

### A. Preliminary cytotoxicity assay

No preliminary cytotoxicity assay was performed.

### B. Mutagenicity assay

Results from the standard plate assay, called Phase 1, are presented in Appendix Table 1 (MRID 43678146, pp.16-17). Results from the pre-incubation assay, called Phase 2, with and without S9-mix are presented in Appendix Tables 2 and 3 (MRID 43678146, pp.18-19) respectively. There was no evidence in either Phase 1 or 2 that ICIA5504 significantly increased the number of revertants over solvent control values in any strain at any concentration tested, either with or without S9-mix. ICIA5504 precipitated on the 5000 µg plates in Phase 1 and on the 5000 and 2500 µg plates with S9-mix in Phase 2. The background lawn was sparse on the 5000 µg plates in phase 1 and on the 5000 µg plates without S9-mix in Phase 2. Positive and solvent control values were appropriate in both studies. Control data are presented separately for Phase 1 in Appendix Table 4 (MRID 43678146, pp.20-23) and for Phase 2 in Appendix Tables 5 (with S9-mix) and 6 (without S9-mix) (MRID 43678146, pp.24-25 and pp.26-7).

## III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This study is acceptable. ICIA5504 was tested to a sufficiently high concentration, experimental protocol was appropriate and solvent and positive control values were as expected in all bacterial strains. There was no evidence of a significant, dose-related increase in the number of revertants per plate in any strain in the



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presence or absence of S9-mix using either the standard plate assay or the pre-incubation assay.

B. STUDY DEFICIENCIES

No deficiencies affecting the acceptability of this study were present.

# APPENDIX

# APPENDIX

Azoxystrobin

Page _____ is not included in this copy.

Pages 9 through 21 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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_____ 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.

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

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