DATA EVALUATION REPORT

7/25/96

AZOXYSTROBIN

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY IN THE MOUSE (84-2)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

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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.

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DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in the mouse
OPPTS 870.5395 [§84-2]

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

TEST MATERIAL (PURITY): E5504 (Azoxystrobin)(97.2% w/w)

SYNONYMS: ICIA5504

CITATION: Jones, K. and J. Mackay (1992) E5504: An evaluation in the mouse micronucleus test. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/P/ 3647, March 6, 1992. MRID 43678148. Unpublished

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

EXECUTIVE SUMMARY: In a C57BL/6JfBL10/Alpk mouse bone marrow micronucleus assay (MRID 43678148), five mice of each sex per harvest time were treated once orally with E5504 (97.2% w/w) at a dose of 5000 mg/kg. Bone marrow cells were harvested at 24 and 48 hours post-treatment. The vehicle was corn oil.

There were no signs of toxicity during the preliminary MTD determination: however, in the main micronucleus test, treated females showed subdued nature, tiptoe gait, piloerection, signs of diarrhoea and signs of urinary incontinence on the day of dosing. No adverse reactions were seen subsequently. The mean percent of polychromatic erythrocytes (PCE) was significantly reduced in treated males at the 48 hour sampling time ($p < 0.01$) but not at 24 hours or at either time in females. The positive control induced the appropriate response. Slides prepared for micronuclei determination were evaluated twice, once as part of the primary study (1000 PCEs/mouse) and again by an independent evaluator (1000 PCEs/mouse from a different area of the slide than that used in the first evaluation). **There was no evidence of an increased induction of micronuclei over solvent control values in bone marrow PCEs in either sex at either sampling time in either evaluation.**

This study is classified as an acceptable study. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: E5504

Description: light brown solid
Lot/Batch #: P39; D7534/22
Purity: 97.2% w/w
Stability of compound: responsibility of sponsor
CAS #: not provided
Solvent used: corn oil

2. Control materials

Negative/Route of administration: none

Vehicle/Final volume/Route of administration: corn oil/20 mL/kg/oral

Positive/Final dose(s)/Route of administration: cyclophosphamide/65 mg/kg/oral

3. Test compound administration

Volume of test substance administered: 20 mL/kg

Route of administration: oral

Dose levels used: 5000 mg/kg in both a preliminary toxicity assay (Phase I)and in the main micronucleus assay (Phase II)

4. Test animals

a. Species: mouse Strain C57BL/6JfBL10/Alpk Age 10-12 weeks in Phase I and 9-12 weeks in Phase II
Weight: male 20.0-26.0g female 15.4-20.6g
Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

b. No. animals used per dose: 5 males 5 females

c. Properly maintained? YES

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B. TEST PERFORMANCE

1. Treatment and Sampling Times

The same for Test compound, vehicle control and positive control
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr __x__ 24 hr __x__ 48 hr _____ 72 hr (mark all that are appropriate), _____ other (describe):

2. Tissues and Cells Examined

__x__ bone marrow _____ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 1000 plus an additional 1000 in a separate evaluation of the same slides
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: 1000 erythrocytes counted to determine PCE/NCE ratio

3. Details of slide preparation

Mice were killed by carbon dioxide inhalation followed by cervical dislocation at 24 or 48 hours post-treatment. Femurs were removed, cleaned of adhering tissue and the iliac end removed. A fine paint brush, rinsed in saline and wetted with a solution of albumin (6% w/v in physiological saline), was dipped into the marrow canal and two smears painted on a labeled clean, dry microscope slide. The procedure was repeated resulting in four marrow smears per slide. The brush was rinsed in physiological saline and reused between animals of the same group; however, to avoid cross-group contamination, separate brushes and containers of physiological saline were used between groups. Slides were air-dried and stained with polychrome methylene blue and eosin using an Ames Hema-Tek staining machine. Slides were coded and scored blind.

4. Statistical methods

The incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample, were considered by analysis of variance, regarding each combination of sampling time, dose level and sex as a separate group. The results were examined to determine whether any differences between vehicle control and E5504 treated groups were consistent between sexes and across sampling times. Values for micronucleated PCEs were trans-

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formed using a natural logarithmic transformation before analysis.

Group means were provided by the least square means but standard means were presented. Each treatment group mean was compared with the concurrent vehicle control group mean using a one-sided Student's t-test based on the error mean square in the analysis.

5. Evaluation Criteria

Micronuclei identification was as described by Schmid (1976):

1. Spherical (or rounded) with well-defined edges
2. Diameters of not less than approximately 1/20 of a polychromatic erythrocyte diameter
3. Dark purple/dark blue staining
4. Lie in the same plane as the polychromatic erythrocyte in which it is contained (determined by focusing)

II. REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY

Five males and 5 females were each given a single oral dose of 5000 mg/kg E5504 in corn oil. No adverse effects of treatment were seen during the 4 day post-treatment observation period. As 5000 mg/kg is an acceptable limit dose, it was selected as the upper dose for the micronucleus assay.

B. MICRONUCLEUS ASSAY

Although no adverse effects of treatment were seen in the preliminary toxicity assay, treated females in the micronucleus assay showed adverse clinical signs including subdued nature, tiptoe gait, piloerection, diarrhoea and urinary incontinence on the day of dosing but not on subsequent days. A statistically significant decrease in the percentage of PCEs, compared to solvent controls, was seen in treated males at the 48 hour sampling time but not at 24 hours or at either sampling time in treated females. Summary results are shown in Appendix Tables 1 and 2 (MRID 43678148, pp 21 and 22). Three females receiving corn oil were killed at 4 hours post-treatment after showing clinical signs suggestive of overdosing.

Two independent evaluations of the slides prepared for micronuclei determinations were performed. No statistically significant increases in the mean incidence of micronucleated PCEs, over solvent control values, were seen in either sex at either sampling time. Summary results of the first evaluation are presented in Appendix

Tables 3 and 4 (MRID 43678148, pp. 19 and 20) and results of the second evaluation are presented in Appendix Tables 5 and 6 (MRID 43678148, pp. 12 and 13 of the first supplement to CTL/P/3647).

Positive and solvent control values were acceptable.

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

A. This study was acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data. Control data were appropriate and the test material was tested to an acceptably high dose. As tested in this study, there was no indication that E5504 induced micronuclei in bone marrow PCEs.

B. STUDY DEFICIENCIES

There were no deficiencies that compromised the acceptability of the study.

References

Schmid, W. (1976). The Micronucleus Test for Cytogenetic Analysis. In: A. Hollaender (Ed). Chemical Mutagens: Principles and Methods for Their Detection. Vol 4, Plenum, New York 31-43.

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APPENDIX

Azoxystrobin

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Pages 8 through 13 are not included in this copy.

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