

DATA EVALUATION REPORT

C12115

AZOXYSTROBIN

STUDY TYPE: IN VITRO MAMMALIAN CYTOGENETICS ASSAY
IN HUMAN LYMPHOCYTES (84-2)

7/25/94

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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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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AZOXYSTROBIN

IN VITRO CHROMOSOMAL ABERRATION (84-2)

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DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics assay in human lymphocytes OPPTS 870.5375 [§84-2].

DP BARCODE: D218319

SUBMISSION CODE: S489692

P.C. CODE: 128810

TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): E5504 (Azoxystrobin) (95.2% w/w)

SYNONYMS: none provided

CITATION: Fox, D. and J. Mackay (1992) E5504: An evaluation in the in vitro cytogenetic assay in human lymphocytes: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report number, CTL/P/3607, January 27, 1992. MRID 43678147. Unpublished.

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

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics chromosomal aberration assay (MRID 43678147), cultures of primary human lymphocytes from one male and one female donor were exposed to E5504 (95.2% w/w) in DMSO at concentrations of 0.5, 1, 5, 10, 20, 30, 40, and 50 $\mu\text{g/mL}$ in the absence of an exogenous metabolic activation system (S9-mix) and to 1, 5, 25, 50, 75, 100, 200, and 300 $\mu\text{g/mL}$ in the presence of S9-mix. The S9 preparation was obtained from Aroclor 1254 induced male rat liver.

E5504 was tested up to cytotoxic concentrations as determined by reduced mitotic index or virtual absence of metaphase cells at the highest concentrations tested. Cultures exposed to E5504 at concentrations of 25, 100 and 200 $\mu\text{g/mL}$ in the presence of S9-mix were evaluated for chromosomal aberrations for both cell donors at the 72 hour harvest time. Cultures exposed to concentrations of 1, 10 and 20 $\mu\text{g/mL}$ and to concentrations of 5, 20 and 50 $\mu\text{g/mL}$ in the absence of S9-mix were evaluated for chromosomal aberrations at 72 hours for the male and female donors respectively. Cultures, from the female donor only, exposed to 20 $\mu\text{g/mL}$ test material in the absence of S9-mix and to 200 $\mu\text{g/mL}$ in the presence of S9-mix were also evaluated at 96 hours. At the 72 hour harvest time in the absence of S9-mix, the mean percent of aberrant cells (excluding gaps in all cases) was 4.5% at 20 $\mu\text{g/mL}$ in cultures from the male donor compared to 0.0% in the solvent control ($p < 0.01$). Comparable values in cultures from the female donor were 4.50% at 5 $\mu\text{g/mL}$

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($p < 0.05$), 8.00% at 20 $\mu\text{g/mL}$ ($p < 0.01$) and 6.00% at 50 $\mu\text{g/mL}$ ($p < 0.01$) compared to the solvent control value of 1.00%. In the presence of S9-mix, the mean percent of aberrant cells was significantly increased ($p < 0.01$) at 200 $\mu\text{g/mL}$ in cultures from both the male and female donor (11.50% vs 0.00% in solvent control and 6.50% vs 0.50% in solvent control respectively). The mean percent of aberrant cells was also significantly increased ($p < 0.05$) at 100 $\mu\text{g/mL}$ in cultures from the male donor (2.50% vs 0.00%). No significant increase in mean percent of aberrant cells was seen, with or without S9-mix, at the 96 hour harvest time. Aberrations were virtually all breaks and fragments or minutes. Positive and solvent controls induced the appropriate response. **There was evidence of a concentration related induction of chromosomal aberrations over background in the presence of moderate to severe cytotoxicity.**

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* 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: off-white solid
Lot/Batch #: P32/A1016/34
Purity: 95.2% w/w
Stability of compound: responsibility of sponsor
CAS #: unknown
Structure: not provided
Solvent used: DMSO
Other comments:

2. Control materials

Negative: growth medium
Solvent/final concentration: DMSO/5 $\mu\text{L/mL}$
Positive/final concentration:
Nonactivation - Mitomycin C/0.2 $\mu\text{g/mL}$
Activation - Cyclophosphamide/50 $\mu\text{g/mL}$

3. Activation

S9 derived from

<input checked="" type="checkbox"/> Aroclor 1254	<input checked="" type="checkbox"/> induced	<input checked="" type="checkbox"/> rat	<input checked="" type="checkbox"/> liver
<input type="checkbox"/> phenobarbital	<input type="checkbox"/> non-induced	<input type="checkbox"/> mouse	<input type="checkbox"/> lung
<input type="checkbox"/> none	<input type="checkbox"/> hamster	<input type="checkbox"/> other	
<input type="checkbox"/> other		<input type="checkbox"/> other	

Male Charles River CD rats were used.

Describe S9 mix composition:

Final concentration in S9-mix (dissolved in deionized water, final pH = 7.4)

Na ₂ HPO ₄	75 mM
KCl	25 mM
Glucose-6-phosphate	4 mM
NADP, sodium salt	3 mM
MgCl ₂	6 mM

4. Test compound concentrations used

Preliminary cytotoxicity test: 2.4, 12, 60, 300, 1500 µg/mL with and without activation

Main mutagenicity test:

without S9-mix:	1, 10, 20 µg/mL (cells from male)
	5, 20, 50 µg/mL (cells from female)
with S9-mix	25, 100, 200 µg/mL (both sexes)

5. Test cells

Mammalian cells in culture - human lymphocytes from one male and one female donor. Peripheral blood was obtained by venepuncture on the days that cultures were to be initiated from healthy non-smoking donors with a known low incidence of chromosomal aberrations in their peripheral blood lymphocytes. Cultures were started by adding 0.5 mL of whole blood and 0.5 mL of a phytohaemagglutinin solution to 9 mL of RPMI-1640 (Dutch modification) medium supplemented with 10% fetal bovine serum, 1.0 IU/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin.

Properly maintained? Y

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay

A preliminary cytotoxicity assay was conducted on blood from both a male and a female donor. E5504 concentrations of 2.4, 12, 60, 300 and 1500 µg/mL were tested with and without S9-mix. About 48 hours after the cell cultures were initiated, the growth medium was replaced with fresh medium and the appropriate concentration of E5504 or solvent control was added. Treatment in the absence of S9-mix continued for the remaining 24 hours of the 72 hour incubation period. Cultures with S9 mix (200 µl of a 1:1 mix of S9 and co-factor solution added at the same time as test agent) were exposed to the test agent for three hours at which time the growth medium was changed and incubation continued for the remainder of the 72 hour culture period. Detailed results from the preliminary cytotoxicity assay were not presented; however, the authors stated that few or no metaphases were seen on slides from both donor cultures treated with 60, 300 or

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1500 $\mu\text{g/mL}$ in the absence of S9-mix or at 300 or 1500 $\mu\text{g/mL}$ in the presence of S9-mix. Mitotic indices, if determined, were not reported.

2. Cytogenetic assay

a. Cell treatment

Cells exposed to test compound, solvent, or positive control for 24 hours (nonactivated), 3 hours (activated)

b. Spindle inhibition

Inhibitor used/concentration: colcemid / 0.4 $\mu\text{g/mL}$
Administration time: 2 hours (before cell harvest)

c. Cell harvest

Cells exposed to test material, solvent or positive control were harvested 0 hours after termination of treatment (nonactivated), 21 hours after termination of treatment (activated). An additional harvest time, 24 hours after the first, was used for cultures from the female donor.

- d. Details of slide preparation: Cultures were transferred to conical centrifuge tubes and cells collected by centrifugation at 400 g for 5 min. Cells were resuspended in 5 mL of 0.075 M KCl at room temperature, an additional 5 mL of the KCl solution was added and the resuspension allowed to stand at room temperature for 10 min. The cells were again pelleted by centrifugation at 400 g for 5 min, the supernatant discarded and the cells fixed in freshly prepared methanol/glacial acetic acid fixative (3:1 v/v) added dropwise with the final volume made up to 10 mL. The fixation procedure was repeated twice. Single drops of cell suspensions were dropped onto clean, moist slides and the slides air dried. The slides were stained in a filtered 10% solution of buffered Giemsa stain (Gurr's R66) in double deionized water for 7 min, rinsed in water, air-dried and mounted in DPX.

e. Metaphase analysis:

No. of cells examined per culture 1000 for mitotic determination and 100 metaphase cells per culture for structural chromosomal aberration determination. Duplicate cultures were used for each test point.

Scored for structural: Y

Scored for numerical: N

Coded prior to analysis: Y

- f. Evaluation criteria: Cells were evaluated for structural chromosomal damage according to the criteria recommended by Scott et al. (1990). The following aberrations were recorded: gaps, breaks, fragments and minutes, multiple damage, interchanges, rearrangements and "others".
- g. Statistical analysis: Data were evaluated for statistical significance at $p < 0.05$ and < 0.01 , using Fisher's Exact Test (one sided).

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

E5504 concentrations of 2.4, 12, 60, 300 and 1500 $\mu\text{g/mL}$, with and without S9-mix, were tested in the preliminary cytotoxicity assay. Few details were presented but presumably the harvest time was 72 hr after culture initiation with exposure to the test material during the last 24 hours in the absence of S9-mix and for 3 hr starting 24 hr before harvest time in the presence of S9-mix. Raw data from the preliminary assay were not reported; however, the authors state that "few or no metaphases were observed on the slides from male and female donor cultures treated at the 60, 300 or 1500 $\mu\text{g/mL}$ concentrations in the absence of S9-mix and at the 300 and 1500 $\mu\text{g/mL}$ concentrations in the presence of S9-mix".

B. CYTOGENETIC ASSAY

In the main cytogenetic assays, E5504 was tested at concentrations of 0.5 through 50 $\mu\text{g/mL}$ in the absence of S9-mix and 1 through 300 $\mu\text{g/mL}$ in the presence of S9-mix. Effects of treatment on mitotic index at the 72 hr harvest time without and with S9-mix are presented in Appendix Tables 1 and 2 respectively (MRID 43678147, pp. 21 and 22). Appendix Table 3 (MRID 43678147, p23) contains the mitotic index results for the 96 hr harvest time (cultures from the female donor only, test material concentrations of 20, 30, 40 and 50 $\mu\text{g/mL}$ in the absence of S9-mix and 75, 100, 200, and 300 $\mu\text{g/mL}$ in the presence of S9-mix). In the absence of S9-mix, the mitotic index was reduced, compared to solvent controls, by approximately 70% or more in cells from both the male and female donor at concentrations of test material 20 $\mu\text{g/mL}$ and greater at the 72 hr harvest time. In the presence of S9-mix, the mitotic index was reduced about 64% in cultures from male donors and about 71% in those from female donors treated at 200 $\mu\text{g/mL}$ and harvested at 72 hr. Cultures from the female donor were also harvested at 96 hr. No significant reduction in mitotic index was seen in the absence of S9-mix at 20 or 50 $\mu\text{g/mL}$, the only two concentrations evaluated; however, the mitotic index was reduced by half at 200 $\mu\text{g/mL}$ in the presence of S9-mix.

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At the 72 hour harvest time, the mean percent of cells with chromosomal aberrations (excluding cells with gaps only) in cultures treated in the absence of S9-mix was significantly increased over the solvent control value at 20 $\mu\text{g/mL}$ in cultures from the male donor and at 5, 20 and 50 $\mu\text{g/mL}$ in cultures from the female donor. In the presence of S9-mix, cultures from both the male and female donor showed significant increases in mean percent of aberrant cells at 200 $\mu\text{g/mL}$. A slight, but statistically significant increase in mean percent of aberrant cells was also seen at 100 $\mu\text{g/mL}$ in cultures from the male donor. No significant increase in mean percent of aberrant cells was seen with or without S9-mix at the 96 hour harvest time. The chromosomal aberrations seen were virtually all breaks or fragments and minutes (gaps were excluded). Chromosomal aberration data are summarized in Appendix Tables 4-7 (MRID 43678147, pp. 24-27).

The positive and solvent control values were appropriate.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

- A. This study was acceptable. The test material was tested to a concentration limited by cytotoxicity with dose-related reductions in mitotic activity and few metaphases present at the highest concentrations tested. The positive controls, Mitomycin C in the absence of S9-mix and Cyclophosphamide in the presence of S9-mix, and the solvent control gave the expected results. Experimental protocol basically followed the pertinent guidelines.

B. STUDY DEFICIENCIES

There were no major deficiencies in this study.

References

Scott, D., Danford, N.D., Dean, B.J., and Kirkland, D.J. (1990). Metaphase Chromosome Aberration Assays in vitro: In: Kirkland, D.J. (Ed) "Basic Mutagenicity Tests: UKEMS Recommended Procedures". Cambridge University Press, Cambridge, pp 62-86.

APPENDIX

APPENDIX

Azoxystrobin

Page _____ is not included in this copy.

Pages 10 through 16 are not included in this copy.

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