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

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008395

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: 041701. Fonofos. Review of Submitted In Vitro Cytogenetics Assay for Reregistration

Tox. Chem. No. 454B
Project No. 1-1092

TO: Joanne Edwards
Special Review and
Reregistration Division (H7508C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 5/16/91
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C) Roger L. Gardner 5/31/91 NB 6/3/91

Record No(s). S394488

Background and Request:

ICI Agricultural Products has submitted an In Vitro Cytogenetic Assay in Human Lymphocytes in partial fulfillment of the mutagenicity requirements for reregistration of fonofos. The Toxicology Branch (TB-I) has been requested to review the study and determine whether or not the study fulfills the requirement for a chromosomal aberration study.

Toxicology Branch Response:

The Toxicology Branch has reviewed the study and has found that it is an acceptable study. The study adequately fulfills the requirement for a chromosomal aberration study for reregistration. No further studies in this category are required at this time.

Fonofos was tested for potential to induce chromosomal aberrations in an in vitro assay in human lymphocytes up to cytotoxic levels. The dose levels tested were 10, 50 and 100 ug/ml both with and without metabolic activation. Fonofos did not induce a significant increase in chromosomal aberrations under the conditions of the assay. Positive controls verified the sensitivity of the assay.

Guideline Series 84: **MUTAGENICITY**

Reviewed By: Pamela M. Hurley, Ph.D. Pamela M. Hurley 5/15/91
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Roger L. Gardner, Head R. L. 6/3/91
Section I, Tox Branch, (H7509C)
Date: 5/15/91

DATA EVALUATION REPORT

CHEMICAL: Fonofos

Tox. Chem. No.: 454B

STUDY TYPE: Mammalian cells in culture cytogenetics assay in human lymphocytes

ACCESSION NUMBER: 418371-01

SYNONYMS/CAS No.: Dyfonate

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

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Fonofos: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes

AUTHOR(S): N. James and J. M. Mackay

STUDY NUMBER(S): SV0481; Report No. CTL/P/3263

REPORT ISSUED: 3/13/91

CONCLUSION(S) - Executive Summary: Fonofos was tested for potential to induce chromosomal aberrations in an in vitro assay in human lymphocytes up to cytotoxic levels. The dose levels tested were 10, 50 and 100 ug/ml both with and without metabolic activation. Fonofos did not induce a significant increase in chromosomal aberrations under the conditions of the study. Positive controls verified the sensitivity of the assay.

Classification: Acceptable

TESTING GUIDELINE SATISFIED: 84-2

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A. MATERIALS

1. Test Material: Name: o-ethyl s-phenyl ethylphosphonodithioate
Description (e.g. technical, nature, color, stability): brown liquid
Batch #: WRC# 11825-25; Sponsor Ref: AI/90/0163; CTL Ref: YO2743/003 Purity: 94.9%
Contaminants: None reported
Solvent used: Dimethylsulfoxide (DMSO)
Other comments: None

2. Control Materials:
Negative: DMSO
Solvent/final concentration: 5 ul/ml
Positive: Non-activation (concentrations, solvent): mitomycin C, 1.0 ug/ml in sterile double deionized water.

Activation (concentrations, solvent): Cyclophosphamide, 50 ug/ml in sterile double deionized water.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

If other, describe below

Describe S9 mix composition (if purchased, give details): S9 buffer: sucrose (250 mmol, tris base (50 mmol, EDTA tetrasodium salt (dihydrate) (1 mmol). Co-factor solution: Na_2HPO_4 (75 mmol), KCl (25 mmol), Glucose-6-phosphate (4 mmol), NADP (Na salt) (3 mmol), $MgCl_2$ (6 mmol).

4. Test compound concentrations used:

Non-activated conditions: 10, 50 and 100 ug/ml

Activated conditions: 10, 50 and 100 ug/ml

5. Test Cells: mammalian cells in culture
Describe cell line, cell strain or primary cell culture (if human lymphocytes, describe conditions of subjects) used: Human peripheral blood was obtained by venepuncture on the day of culture initiation from 2 healthy non-smoking human donors, donor 1 being male and donor 2 being female. Both donors have a previously established low incidence of chromosomal damage in their peripheral blood lymphocytes.

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound for:
__3__ hours (non-activated) __3__ hours (activated)

b. Cells exposed to positive controls for:
__3__ hours (non-activated) __3__ hours (activated)

c. Cells exposed to negative and/or solvent controls for:
__3__ hours (non-activated) __3__ hours (activated)

2. Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; if lymphocytes, nature of mitogen and when added; cell density during treatment; harvest times; spindle inhibitor and when used; chromosome preparation and analysis; number of cells/culture analyzed; statistics used): The cultures were established with supplemented RPMI 1640 tissue culture medium and the lymphocytes were stimulated to enter cell division by phytohemagglutinin (PHA). The cultures were maintained at 37°C for 48 hours. After that time, aliquots of the test material solutions and solvent and positive control solutions were administered. The S-9 mix was added to the appropriate cultures as well. The cultures were then incubated at 37°C for 3 hours, after which the growth medium was replaced with fresh medium and the cultures were re-incubated for the remainder of the 72-hour growth period. Treatment with colcemid (0.4 ug/ml) at 70 hours arrested dividing lymphocytes. The cells were harvested at 72 hours, subjected to hypotonic treatment with 0.075 M KCl at room temperature, fixed in methanol/glacial acetic acid and dropped as single drops of cell suspension onto clean, moist slides which were then stained in Giemsa and mounted in DPX.

The mitotic index of each slide was assessed by examining 1000 lymphocytes/culture and calculating the % of cells in metaphase. One hundred cells in metaphase were analyzed from each selected culture for the incidence of structural chromosomal damage. The total number of abnormal cells, excluding those with only gap-type aberrations, for each of the treatment groups was compared to the appropriate solvent control values using the Fisher's Exact Test (one sided).

3. Preliminary cytotoxicity assay (include concentration ranges activation and nonactivation; reported results, e.g. cytotoxicity and solubility; rationale for determining harvest times (e.g. alterations in cell cycle) and concentration levels, if reported): An initial range-finding cytotoxicity study was conducted with blood from each of the two donors, both with and without metabolic activation. The cultures were treated with a range of 5 concentrations of fonofos: (ranging from 8 to 5000 ug/ml. An additional cytotoxicity study was conducted using a narrower range of 7 concentrations, ranging from 10 to 250 ug/ml. At 200, 1000 and 5000 ug/ml, the test material precipitated. Either very few or no metaphases were observed in any of these cultures. In the second cytotoxicity assay, dose-related reductions in mitotic activity were seen in cultures from both donors, both with and without metabolic activation. At 100 ug/ml, reductions in mean mitotic activity when compared to controls (without activation) were approximately 75% for the male donor and 79% for the female donor. With activation, the reductions in mitotic activity at the same dose level were 53% for the male donor and 39% for the female donor. Based on the results of this test, concentrations of 10, 50 and 100 ug/ml were selected for the main assay, both with and without activation. See tables 1 and 2 for summary tables of the preliminary cytotoxicity assay.

4. Cytogenetics assay (reported results, e.g. induction of aberration frequency; types of aberrations, e.g. whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; number of cultures per concentration; levels of cytotoxicity obtained, e.g. effect on mitotic index or cell survival, if examined; include representative table, if appropriate): Tables 3 and 4 summarize the results of the main cytogenetics assay. No statistically or biologically significant increases in chromosomal aberration frequencies (including or excluding gap-type aberrations) were observed in cultures from either donor, either in the presence or absence of metabolic activation. The positive controls indicated the appropriate responses in the test system, verifying its sensitivity. It should be noted that for the positive controls, the mean mitotic indices indicate that the dose levels selected were moderately toxic to the cells (mitotic activity when compared to controls were reduced by 58.8, 72.7, 9.4 and 53.35 percent for the single cultures reported. The first two numbers are

for mitomycin C and the second two numbers are for cyclophosphamide).

5. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions; remember, do not include gaps in final aberration frequency analysis): This study appears to be acceptable and complete as written. The test chemical does not appear to induce chromosomal aberrations under the conditions of the study. The test chemical was tested up to cytotoxic levels.

6. Was test performed under GLPs (is a quality assurance statement present)? Yes

7. CBI appendix attached? No

Page _____ is not included in this copy.

Pages __7___ through __10__ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

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_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

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