



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

CAS

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 041701. Fonofos. Data Call-In. Review of Submitted
Mutagenicity Studies.

Tox. Chem. No. 454B
Project No. 1-0952

TO: Joanne Edwards
PM Team # 74
Special Review and
Reregistration Division (H7508C)

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

Pamela M. Hurley 5/29/91

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C)

Roger L. Gardner

11-7-91

Record No(s). S393672

KB 11/5/91

Background and Request:

In compliance with the registration requirements for the Fonofos Registration Standard, ICI submitted two mutagenicity studies, one to satisfy the requirement for a test for gene mutations and one to satisfy the requirement for a test for other genotoxic effects. The Toxicology Branch has been asked to examine the two studies and determine whether or not they fulfill the regulatory requirements for these two studies.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has examined the two submitted mutagenicity studies and has determined that they satisfy the regulatory requirements for a gene mutation study and for a test for other genotoxic effects.



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In the first study, Fonofos was tested for potential to induce reverse mutations in Salmonella typhimurium, both with and without metabolic activation at the following dose levels: 0.32, 1.6, 8.0, 40, 200, 1000 and 5000 ug/plate. Fonofos was tested up to levels of cytotoxicity. It did not induce a significant increase in the number of reverse mutations when compared to the vehicle control, DMSO and to the absolute control. The study is classified as acceptable.

In the second study, Fonofos was tested in a mouse micronucleus test at 6 and 9.5 mg/kg. There were no statistically or biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in mice treated with fonofos at either dose level at any of the sampling times investigated, when the data from both sexes were considered separately or when combined (when compared to vehicle control values). The percentage of polychromatic erythrocytes in the treated animals when compared to the controls indicates that there was some indication of cytotoxicity to the bone marrow cells at the dose levels tested. The study is classified as acceptable.

008808

Guideline Series 84: **MUTAGENICITY**

Reviewed by: Pamela M. Hurley, Ph.D. *Pamela M. Hurley* 5/29/91
Section I, Tox Branch, (H7509C)
Secondary Reviewer(s): Irving Mauer, Ph.D. *Irving Mauer* 5/29/91
Toxicology Branch, (H7509C)
Roger L. Gardner, Head, Section I, Tox Branch, (H7509C) *R. L. Gardner* 11/4/91
Date: May 23, 1991

DATA EVALUATION REPORT

CHEMICAL: Fonofos

Tox. Chem. No.: 454B

STUDY TYPE: In vivo micronucleus assay in the mouse

ACCESSION or MRID NUMBER: 418133-01

SYNONYMS/CAS No.: Dyfonate

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

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

TITLE OF REPORT: Fonofos: An Evaluation in the Mouse Micronucleus Test

AUTHOR(S): K. Jones, J.M. Mackay

STUDY NUMBER(S): SMO365, Report No. CTL/P/2827

REPORT ISSUED: 1/17/90

CONCLUSION(S) - Executive Summary: Fonofos was tested in a mouse micronucleus test at 6 and 9.5 mg/kg. There were no statistically or biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in mice treated with fonofos at either dose level at any of the sampling times investigated, when the data from both sexes were considered separately or when combined (when compared to vehicle control values). The percentage of polychromatic erythrocytes in the treated animals when compared to the controls indicates that there was some indication of cytotoxicity to the bone marrow cells at the dose levels tested.

Classification: Acceptable

TESTING GUIDELINE SATISFIED: 84-2

A. MATERIALS

1. Test Material: Name: Fonofos (o-ethyl s-phenyl ethylphosphonodithioate)
Description (e.g. technical, nature, color, stability):
Technical, brown liquid, specific gravity 1.15

Batch #: Lot 11825-25 Ex WRC, CTL Reference # YO2743/003/001,
Sponsor # SC 12/89 Purity: assumed 100%
Solvent used: Corn oil

2. Control Materials:

Vehicle control route of administration: orally by gavage

Vehicle - final volume: 10 ml/kg

Positive control/Final dose(s)/Route of administration:
cyclophosphamide 65 mg/kg in physiological saline dosed orally
in a volume of 10 ml/kg.

3. Test compound:

Volume of test substance administered: 10 ml/kg

Route of administration: orally in corn oil

Dose levels used: 6 and 9.5 mg/kg

4. Test animals:

a. Species mouse Strain C57BL/6JfCD-1/Alpk Age 10-14 wks
(phase I) 10-12 wks (phase II)

Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals,
Alderley Park, Macclesfield, Cheshire, England

b. No. animals used per dose: For phase I part a, two males
per dose were used. For phase I part b, 5 males and 5 females
per dose were used. For phase II, 15 animals per sex per dose
level were used, including vehicle controls. Five per sex per
dose were sacrificed for each of 3 time periods. For positive
controls, only one dose level was used with one time period.
Therefore, only 5 per sex were used.

c. Properly maintained? Yes

B. TEST PERFORMANCE1. Treatment and Sampling Times:a. Test compound

Dosing: ☒ once ☐ twice (24 hr apart)
☐ other (describe):

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

b. Negative and/or vehicle control

Dosing: ☒ once ☐ twice (24 hr apart)
☐ other (describe):

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

c. Positive control

Dosing: ☒ once ☐ twice (24 hr apart)
☐ other (describe):

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

2. Tissues and Cells Examined:

☒ bone marrow ☐ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal:
1000

No. of normochromatic erythrocytes (NCE; more mature RBCs)
examined per animal: The ratio of polychromatic to
normochromatic erythrocytes was determined (1000 cells were
counted)

3. Details of slide preparation: Animals were killed at 24, 48
or 72 hours after receiving a single dose of test material.
The femurs were removed and stripped clean of muscle. The
iliac end of the femur was removed and using a fine paint
brush and saline, smears of the bone marrow were made on clean
slides. When dried, the slides were stained with polychrome
methylene blue and eosin. They were coded and scored blind.

4. Preliminary cytotoxicity assay (reported results, e.g. include dose range, signs of toxicity - e.g. MTD considerations, clinical signs; no. animals): There was no official preliminary cytotoxicity assay. Instead, the study was divided into two phases. The first phase consisted of determination of a median lethal dose calculated on the deaths over a four-day observation period (MLD) using a single oral dose. In part a of Phase I, Groups of 2 male animals were dosed with fonofos at dose levels of 5, 10, 20 and 50 mg/kg. At the 3 higher dose levels, all animals either died or were killed in extremis. No animals died at the 5 mg/kg dose level. In part b of Phase I, groups of 5 male and 5 female animals were dosed with either 5, 7.5, 10 or 20 mg/kg fonofos. No clinical signs of toxicity were reported. The following mortalities were reported: 5 mg/kg: 0/5 males, 0/5 females; 7.5 mg/kg: 0/5 males, 0/5 females; 10 mg/kg: 2/5 males, 1/5 females; 20 mg/kg: 5/5 males, 5/5 females. The report stated that "from the mortalities, the median lethal dose (MLD) over a 4 day observation period was estimated by logistic regression as 11.9 mg/kg with the 50% and the 80% MLD levels being calculated as 6 mg/kg and 9.5 mg/kg respectively."
5. Micronucleus assay (reported results, e.g. include induction of micronuclei; appropriateness of negative, solvent and positive control micronucleus frequencies; ratio of PCE/NCE; sex differences (if any); appropriateness of dose levels and route; statistical evaluation; include representative table, if appropriate): The following clinical signs of toxicity were observed: males at 6 mg/kg - subdued nature, hunched posture and tremors (the latter in a single animal only); females at 6 mg/kg - no adverse reactions to treatment; females at 9.5 mg/kg - subdued and exhibited a hunched posture in the first 24-hour period post-dose (two females were killed in extremis approximately 5 1/2 and 27 1/2 hours post-dose respectively); males at 9.5 mg/kg - subdued nature, hunched posture, tremors, irregular breathing, salivation, piloerection, signs of urinary incontinence and reduced body temperature (8 males were killed in extremis during the 24 hour period post-dose).

The results of the micronucleus test indicated that there were no statistically or biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in mice treated with fonofos at either dose level at any of the sampling times investigated, when the data from both sexes were considered separately or when combined (when compared to vehicle control values, see tables 1-3). The positive control gave appropriate positive responses which validated the sensitivity of the system (increases between approximately 10 and 14 times the vehicle control values, see tables 1-3). In

MICRONUCLEUS

the treated animals there was a statistically significant reduction in the percentage of polychromatic erythrocytes when compared to controls in male mice at both dose levels (see tables 4-6). The authors stated that these decreases were seen in male mice 24 hours after being dosed at the 50% and 80% MLD levels. They stated that "these reductions are significant when compared to the 24 hour male vehicle controls but the mean values recorded for the fonofos treated animals are similar to those recorded for the male vehicle controls at 48 and 72 hours post-dose. Comparison of the individual animal values however shows that the fonofos treated animals have uniformly low values for the percentage of polychromatic erythrocytes, whereas the male vehicle control values are significantly variable. These data therefore indicate that fonofos or a metabolite has induced a cytotoxic effect causing a depression in bone marrow proliferation." The Toxicology Branch accepts this as a plausible explanation (see Appendix G attached). It appears from the tables that the cytotoxicity of fonofos is greater in the males than in the females (see tables 4-6).

6. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions): The study appears to be acceptable as written. The test compound was tested up to a level of cytotoxicity, particularly in males.
7. Was test performed under GLPs (is a quality assurance statement present)? Yes
8. CBI appendix attached? No

Page ____ is not included in this copy.

Pages __8__ through __14__ are not included in this copy.

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 - ____ Information about a pending registration action.
 - x FIFRA registration data.
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Guideline Series 84: **MUTAGENICITY**

Reviewed by: Pamela M. Hurley, Ph.D. *Pamela M. Hurley* 5/21/91
Section I, Tox Branch, (H7509C)
Secondary Reviewers: Irving Mauer, Ph.D. *Irving Mauer* 5/21/91
Roger L. Gardner, Head, Section I, Tox Branch, (H7509C) *R.L. 11/4/91*
Date: May 20, 1991

DATA EVALUATION REPORT

CHEMICAL: Fonofos

Tox. Chem. No.: 454B

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER: 417692-01

SYNONYMS/CAS No.: Dyfonate

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

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

TITLE OF REPORT: Fonofos - An Evaluation of Mutagenic Potential Using S. Typhimurium

AUTHOR(S): R.D. Callander

STUDY NUMBER(S): YV2906

REPORT NUMBER: CTL/P/3153

REPORT ISSUED: 12/21/90

IDENTIFYING VOLUME: 1

CONCLUSION(S) - Executive Summary: Fonofos was tested for potential to induce reverse mutations in Salmonella typhimurium, both with and without metabolic activation at the following dose levels: 0.32 1.6, 8.0, 40, 200, 1000 and 5000 ug/plate. Fonofos was tested up to levels of cytotoxicity. It did not induce a significant increase in the number of reverse mutations when compared to the vehicle control, DMSO and to the absolute control.

SALMONELLA

Study: Acceptable

TESTING GUIDELINE SATISFIED: 84-2

A. MATERIALS

1. Test Material: Name: Fonofos (o-ethyl s-phenyl ethylphosphonodithioate)
Description (e.g. technical, nature, color, stability): technical; amber/brown liquid; stable under normal storage conditions and under conditions used in this study.
Batch #: 11825-25 Purity: 94.9% w/w
Contaminants: none listed
Solvent used: dimethylsulfoxide (DMSO, 0.1 ml)
2. Control Materials:
Negative: Absolute control and vehicle control (DMSO)
Solvent/final concentration: 0.1 ml
Positive: Non-activation:
Acridine mutagen ICR191 - 0.5, 1.0, 2.0 ug/plate TA 1537
Daunomycin hydrochloride - 0.2, 0.5, 1.0 ug/plate TA98
N-methyl-N'-nitro-N-nitrosoguanidine - 1.0, 2.0, 5.0 ug/plate TA 100
4-Nitro-o-phenylenediamine - 1.0, 2.0, 5.0 ug/plate TA 1538

Activation:
2-Aminoanthracene (2-anthramine) 0.2, 0.5, 1.0, 2.0 ug/plate (all strains)
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	

Describe S9 mix composition (if purchased, give details): Buffer: 250 mM sucrose, 50mM tris base, 1.0 mM EDTA tetrasodium salt (dihydrate). Cofactor solution: Na₂PO₄ 100 mM, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP (Na salt), 8 mM MgCl₂. S-9 mix prepared as follows: 3 ml S9 fraction, 7 ml sucrose-tris-EDTA buffer, 20 ml co-factor solution.

SALMONELLA

4. Test organisms: S. typhimurium strains
___ TA97 x TA98 x TA100 ___ TA102 ___ TA104
x TA1535 x TA1537 x TA1538 ; list any others:
Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation,
R factor)? Yes
5. Test compound concentrations used:
Non-activated conditions: 0.32, 1.6, 8.0, 40, 200, 1000 and
5000 ug/plate

Activated conditions: 0.32, 1.6, 8.0, 40, 200, 1000 and 5000
ug/plate

B. TEST PERFORMANCE

1. Type of x standard plate test
Salmonella assay: ___ pre-incubation (___ minutes)
___ "Prival" modification (i.e. azo
reduction method)
___ spot test
___ other (describe in a.)

Protocol (brief description, or attach copy to appendix, if appropriate; e.g. include mediums used, incubation times, assay evaluation): A copy of the test protocol is attached as an addendum to this document.

2. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g. cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):
There was no preliminary cytotoxicity assay.
3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate): Two experiments were conducted. In the first assay, the test sample was tested over a dose range of 1.6 - 5000 ug/plate, both in presence and absence of metabolic activation. The 5 tester strains listed above were tested. There was toxicity with strains TA1535, TA1537 and TA 100 and these strains were retested in the second assay with lower dose levels while TA1538 and TA98 were retested with the same dose levels. Table 1 gives the results of the first experiment. The background lawn was sparse on the 5000 ug dose plates.

SALMONELLA

In the second assay, tester strains TA1538 and TA98 were tested at the same dose levels as in the first assay. The other 3 strains were tested over a dose range of 0.32 - 1000 ug/plate. Table 2 gives the results of the second experiment. Again, the background lawn was sparse on the 5000 ug dose plates. In addition, the compound precipitated on some of these plates. In both experiments, the compound did not induce any significant, reproducible increases in the observed number of revertant colonies in any of the 5 tester strains, either with or without metabolic activation. The positive controls (data in tables 3 and 4) induced the expected responses, indicating that the strains were responding satisfactorily in each case. The negative and absolute controls (Tables 3 and 4) also gave the appropriate responses.

4. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions): The report appears to be acceptable as written. Fonofos does not appear to induce reverse mutations in Salmonella typhimurium under the conditions of the assay.
5. Was test performed under GLPs (is a quality assurance statement present)? Yes
6. CBI appendix attached No

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Study/Lab/Study #/Date

Accession No.

Material

Results:

LD50, LC50, PIS, NOEL, LEL

TOX

CORE Grade/DOC. NO.

In vivo mouse micronucleus/ ICI Central
Tox. Lab, UK/ 5M0365, CTL/P/2227;
1/17/90

Fonofos
Assumed 100%
purity

418133-01

Fonofos was tested in ~~in vivo~~ at the
following dose levels: 6 and 9.5 mg/kg.
No increases in micronucleated PCE's.
~~Results~~ Indications that it was tested
up to level of cytotoxicity

Acceptable
on

Ames Test/ ICI Central Tox. Lab, UK/
V2906; CTL/P/3153; 12/21/90

Fonofos
94.9% pure
w/w

417692-01

Tested with and without activation
at 0.32, 1.6, 8.0, 40, 200, 1000, 5000
µg/plate. Tested up to levels of
cytotoxicity. Results negatives
when compared to vehicle (DMSO)
and absolute controls.

Acceptable