CAS 454B FILE

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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

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 Pamela M. Hurley 5/29/91
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I Roger L. Gardner 11-4-91
Health Effects Division (H7509C)

Record No(s). S393672 EB 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.

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.

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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. 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 PERFORMANCE

1. Treatment and Sampling Times:
   a. Test compound
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): _____ 6 hr _____ 12 hr
      __x__ 24 hr __x__ 48 hr __x__ 72 hr (mark all that are appropriate)
      _____ other (describe):
   b. Negative and/or vehicle control
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): _____ 6 hr _____ 12 hr
      __x__ 24 hr __x__ 48 hr __x__ 72 hr (mark all that are appropriate)
      _____ other (describe):
   c. Positive control
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): _____ 6 hr _____ 12 hr
      __x__ 24 hr _____ 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
   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

the treated animals there was a statistically significant reduction in the percentage of polychromatic erthrocytes 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 poylchromatic 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

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_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ 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.

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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. [signature] 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.

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:

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ 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: Na_2PO_4 100 mM, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP (Na salt), 8 mM $MgCl_2$. S-9 mix prepared as follows: 3 ml S9 fraction, 7 ml sucrose-tris-EDTA buffer, 20 ml co-factor solution.

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 Salmonella assay:
_x_ standard plate test
___ 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 (concurrentand/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.

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	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
In vivo mouse micronucleus/ ICI Central Tox. Lab, Uk/ SM0365; CTL/P/2827; 1/17/90	Fonofos Assumed 100% purity	418133-01	Fonofos was tested ~~in a manner~~ at the following dose levels: 6 and 9.5 mg/kg. No increases in micronucleated PCE's. ~~Probab~~ Indications that it was tested up to level of cytotoxicity		Acceptable 28
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 ug/plate. Tested up to levels of cytotoxicity. Results negatives when compared to vehicle (DMSO) and absolute controls.		Acceptable