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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 627. 041701. Fonofos. Review of Acute Inhalation Study.

Tox. Chem. No. 454B
Project No. 1-2017

TO: Joanne Edwards, PM Team # 72
Special Review and Reregistration
Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
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Pamela M. Hurley 10/29/91

THRU: Roger L. Gardner, Section Head
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Health Effects Division (H7509C)
Roger L. Gardner 11/1/91 KB 11/05/91

Record No(s). S400967

Background and Request:

ICI Americas, Inc. has submitted an acute inhalation study on Technical Fonofos in response to the Registration Standard. The Toxicology Branch has been asked to review the study and comment.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has reviewed the acute inhalation study on Technical Fonofos and has found it to be acceptable. The study fulfills the regulatory requirement for an acute inhalation study on Technical Fonofos. The following paragraph is a summary of the results of the study.

Fonofos was tested in an acute inhalation study at the following concentrations: 0, 4.75, 33.5 and 77.7 µg/l. The median lethal concentration [MLC] for fonofos, based on the atmospheric concentrations achieved in the study was established to be 51.0 µg/l (33.5, 77.7 confidence limits) for males and 17.9 µg/l (8.6, 37.0 confidence limits) for females. Clinical signs of toxicity and cholinesterase inhibition were evident and were consistent with the combination of neurological and irritancy effects which are typical of those seen following exposure to organophosphorus compounds.

Plasma cholinesterase activity was inhibited by 30% in males at 33.5 µg/l (395 µg/l versus 564 µg/l for controls) at day 2 and by 23% in females at 4.75 µg/l (816 versus 1065 µg/l in controls - not enough surviving males and females in high dose group or females in mid-dose group to calculate values). There was some evidence of a return to the control values by the end of the observation period.

Erythrocyte cholinesterase activity was inhibited by 35% in males at 33.5 µg/l (1223 versus 1890 µg/l) and by 37% in females at 4.75 µg/l (1420 versus 2272 µg/l) at day 2. By the end of the observation period, minimal depressions were present in males . . . (both low and mid-dose groups) and in low dose females.

Brain cholinesterase activity was minimally inhibited in males at 33.5 µg/l (8.7 versus 10.7 µg/l in controls). No significant differences were observed in the low dose group males. There were no significant differences observed in the females.

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Reviewed By: Pamela Hurley, Ph.D. Pamela M. Hurley 10/29/91
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner Roger L. Gardner 11/1/91
Section I, Tox. Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation (81-3) - rat

TOX. CHEM. NO.: 454B

ACCESSION NUMBER/MRID NO.: 419359-01

TEST MATERIAL: Fonofos

SYNONYMS: Dyfonate

STUDY NUMBER(S): HR2047

REPORT NUMBER: CTL/P/3307

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

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

TITLE OF REPORT: Fonofos: 4-Hour Acute Inhalation Toxicity Study in the Rat

AUTHOR(S): Lewis, R.W.; Mould, A.P

REPORT ISSUED: 4/11/91

CONCLUSION: Fonofos was tested in an acute inhalation study at the following concentrations: 0, 4.75, 33.5 and 77.7 µg/l. The median lethal concentration [MLC] for fonofos, based on the atmospheric concentrations achieved in the study was established to be 51.0 µg/l (33.5, 77.7 confidence limits) for males and 17.9 µg/l (8.6, 37.0 confidence limits) for females. Clinical signs of toxicity and cholinesterase inhibition were evident and were consistent with the combination of neurological and irritancy effects which are typical of those seen following exposure to organophosphorus compounds.

Toxicity Category: Toxicity Category I

Classification: Acceptable

Testing Guideline Satisfied: 81-3

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: o-ethyl s-phenyl ethylphosphonodithioate

Description: Amber liquid
Batch #(s), Other #(s): Lot 11825-25 ex WRC; Ref. # Y02743/003
Purity: 94.9% w/w fonofos
Source: ICI Americas, Inc. via ICI Agrochemicals, UK
Vehicle (if applicable): None
Positive Control(s) (if applicable): None

2. Test Animals:

Species and Strain (sexes): Alpk:APpkSD (Wistar-derived) male and female rats
Age: 7-10 weeks
Weight(s): 234-275 g (M); 197-228 g (F)
Source(s): Alderley Park, Cheshire, UK

3. Procedure:

a. Atmosphere Generation: The report stated the following: "Each atmosphere was generated using a glass concentric-jet atomiser and size-selective cyclone. The test substance was pumped to the atomiser using a Gilson peristaltic pump. Clean, dry air...was passed through the atomiser at flow rates of 25 litre/min (Groups 1 and 4) and 15.5 litre/min (Group 3) at normal temperature and pressure and carried the atmospheres to the exposure chambers. The control chamber was also supplied with air at a flow rate of 25 litre/min. Air flow rates were measured using variable area flowmeters, recorded at frequent intervals and were altered as necessary to maintain the target concentrations. Atmospheres of the test material were controlled on the basis of the total particulate concentration."

Particulate Concentrations: Particulate concentrations of the test material close to the animals' breathing zone were measured gravimetrically, at frequent intervals during exposure. The test atmosphere was drawn through a 25 mm diameter Vinyl Metricel (VM-1) filter, at a known flow rate for a known time. The filter was weighed before and after the sample was taken. The aerodynamic particle size of each test

atmosphere was measured by means of a Marple Cascade Impactor. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated.

Analyzed Atmospheric Concentrations: The atmospheric concentration of the test material was determined by dissolving the formulation deposited on the VM-1 filters and the stages of the Cascade Impactor in ethyl acetate and then diluting with ethyl acetate where necessary. The resultant solutions were then analysed by gas chromatography to calculate the atmospheric concentrations.

Exposure System: Animals were exposed nose-only in restraining tubes. The exposure chamber was allowed to reach the target concentration and was required to be stable for approximately 30 minutes prior to exposure. Temperature and relative humidity within each chamber were measured at frequent intervals. The temperature ranged from 18.9-20.9 °C and the relative humidity ranged from 10-19%.

b. Basis for Selection of Dose Levels: Dose levels were selected on the basis of a pretest with several animals.

c. Animal Assignment and Dose Levels:

Test Group	Target Exposure Level (µg/l) Total Particulate Concentration	Number of Animals	
		male	female
1	75	5	5
2	0 (Control)	5	5
3	5	5	5
4	25	5	5

d. Protocol: The animals were housed 5/cage during the observation period. Each group was exposed to the test material for a single 4-hour period and then maintained untreated for a 14-day observation period. The controls were exposed to air only but otherwise treated in a similar manner to the test animals.

e. Clinical Observations and Mortality: All animals were examined prior to the start of the test. During exposure, they were examined frequently. At

the end of the exposure period and once daily thereafter throughout the observation period, each animal was given a detailed clinical examination.

f. Body Weight Determinations: All animals were weighed on days -1, 1, 2, 3, 8 and 15.

g. Clinical Chemistry: Blood was taken on day 2 (tail vein) and at termination (cardiac puncture) from all surviving animals for the analysis of plasma and erythrocyte cholinesterase activities. In addition, brain cholinesterase activity was analyzed from all surviving animals at termination.

h. Terminal Procedure: All surviving animals were anesthetized with halothane BP vapor and killed by exsanguination via cardiac puncture. The rats were then grossly examined with particular attention to the abdominal and thoracic viscera. Lungs (with trachea and larynx attached) were excised, trimmed and weighed (following removal of the larynx). The lungs were then preserved along with any other abnormal tissues for possible microscopic examination. Animals which were killed *in extremis* were examined as soon after death as possible, but organ weights were not recorded.

i. Analyses of Results: Bodyweight gains were calculated from day 1 bodyweight values. Linear log-dose interpolation was used to estimate the median lethal concentration for the male mortality data. Approximate confidence limits are given by the highest dose with no lethalities and the lowest dose with 100% mortality. For female data, the median lethal concentration was estimated by logistic regression. Confidence limits were calculated using a likelihood ratio interval.

B. RESULTS:

1. Atmosphere Analysis: The nominal concentrations from the weight loss of the fonofos technical material were calculated to be as follows:

Group	Target Particulate Concentrations (µg/l)	Nominal Concentration (µg/l)
1	75	740
3	5	1660
4	25	640

Particulate Concentrations: The mean concentrations (+/- standard deviation) were as follows:

Group	Target Particulate Concentration (µg/l)	Measured Particulate Concentration (µg/l) - Mean +/- SD
1	75	77.7 +/- 4.73
3	5	4.75 +/- 2.53
4	25	33.5 +/- 13.1

Analyzed Atmospheric Concentrations: The atmospheric concentrations of the test material as determined by chemical analysis and expressed as a percentage of the total particulate were as follows:

Group	Target Particulate Concentration (µg/l)	Analyzed Fonofos Concentration (µg active ingredient) Mean +/- SD	% Total Particulate
1	75	74.7 +/- 4.32	96.2 +/- 1.4
3	5	3.45 +/- 1.88	72.1 +/- 1.6
4	25	28.5 +/- 11.7	84.2 +/- 3.3

Aerodynamic Particle Size Distribution: The aerodynamic particle size distribution of the total particulate was extrapolated to be as follows:

Group	Particulate Concentration (µg/l)	Median Size (MMAD) µm	Geometric Standard Deviation
1	77.7	0.97	1.95
3	4.75	1.33	1.84
4	33.5	0.96	1.83

The authors stated that similar percentages on the impactor stages were seen with the amounts of fonofos determined by chemical analysis.

2. Clinical Observations and Mortality: There were no deaths in the lowest dose group. Two females died in the mid-dose group and two females were killed immediately following exposure due to severe clinical signs of toxicity. In the high dose group, 1 male and all the females either died or were killed during the exposure period due to severe clinical effects in the highest dose group. The remaining 4 males in the high dose group were killed immediately following exposure

due to severe clinical effects. The median lethal concentration [MLC] for fonofos, based on the atmospheric concentrations achieved in the study was established to be 51.0 µg/l (33.5, 77.7 confidence limits) for males and 17.9 µg/l (8.6, 37.0 confidence limits) for females.

During exposure, the following observations were observed: wet fur (all animals, associated with restraint); chromodacryorrhea (low and mid-dose); stains around the snout (low dose - associated with restraint); lacrimation (all treated animals); auditory hypoesthesia, slow deep breathing, irregular breathing and tail lashing (mid- and high dose groups); fasciculations (mid-dose); and salivation and shaking (high dose group).

Immediately following exposure, the significant clinical signs of toxicity were indicative of irritancy and of a depressant effect on the nervous system. These included lacrimation (mid- and high dose groups); salivation and fasciculations (mid-dose females and high dose males); reduced stability, splayed gait and clonic convulsions (mid-dose females); irregular breathing and prostration (high dose groups); and reduced activity and reflexes and exophthalmos in the high dose groups. Females appeared to be more susceptible to the effects of fonofos than males.

During the observation period the clinical condition of the surviving animals had improved by day 2, although reduced activity, splayed gait, reduced breathing rate, irregular breathing and salivation were still present in the mid-dose group (all the high dose animals were dead by this time). The authors stated that there were no toxicologically significant abnormalities after day 3, although some females in the mid-dose group exhibited decreased activity and signs of urinary incontinence up to and including day 6.

3. Body Weight Determinations: A statistically significant decrease in bodyweight gain was observed in males in the mid-dose group on day 2 when compared to controls. The surviving female in that dose group also showed a significant loss in bodyweight gain up to and including day 8. After that time, the animals gained enough weight such that they were comparable to controls by the end of the observation period.

4. Clinical Chemistry: Plasma cholinesterase activity was inhibited by 30% in the males in the mid-dose group (395 µg/l versus 564 µg/l for controls) at day 2 and by

23% in the females in the low dose group (816 versus 1065 µg/l in controls, not enough surviving females in mid-dose to calculate value). There was some evidence of a return to the control values by the end of the observation period.

Erythrocyte cholinesterase activity was inhibited by 35% in mid-dose males (1223 versus 1890 µg/l) and 37% in low-dose females (1420 versus 2272 µg/l) at day 2. By the end of the observation period, minimal depressions were present in males (both low and mid-dose groups) and in low dose females.

Brain cholinesterase activity was minimally inhibited in males in the mid-dose group (8.7 versus 10.7 µg/l in controls). No significant differences were observed in the low dose group.

5. Gross Pathology: The authors stated that two intercurrent males in the high dose group had red spots on the lungs. At termination, one male in the mid-dose group had red spots on the lung. Another male in the same group had a speckled thymus, which is probably a stress related change. No other significant changes were observed in the treated groups.

6. Organ Weights: No significant differences were observed in lung weights between treated and control animals.

7. Quality Assurance Measures: A signed statement of GLP compliance was provided.

C. DISCUSSION: This was a well-run complete study. It is classified as acceptable. The median lethal concentration [MLC] for fonofos, based on the atmospheric concentrations achieved in the study was established to be 51.0 µg/l (33.5, 77.7 confidence limits) for males and 17.9 µg/l (8.6, 37.0 confidence limits) for females.

Tox. Chem No. 454B File Last Updated _____ Current Date 10/29/91

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation/ICI Central Tox. Lab./#HR2047; CTL/P/3307; 4/11/91	Fonofos 94.9% w/w pure	419359-01	Dose levels tested: 0, 4.75, 33.5 and 77.7 µg/l. Median lethal concentration [MLC] for fonofos, based on the atmospheric concentrations achieved in the study was established to be 51.0 µg/l (33.5, 77.7 confidence limits) for males and 17.9 µg/l (8.6, 37.0 confidence limits) for females. Clinical signs of toxicity and cholinesterase inhibition were evident and were consistent with the combination of neurological and irritancy effects which are typical of those seen following exposure to organophosphorus compounds.	I	Acceptable