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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 28 1985

MEMORANDUM

SUBJECT: Dimethoate, 241-75: Review of Mutagenicity with Dimethoate
Accession No. 257603, Caswell No. 358

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THRU: Robert B. Jaeger, Section Head
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Petitioner:

American Cyanamid Company
Agricultural Research Division
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Recommendation:

The registrant should be apprised the deficiencies reported in the Micronucleus Test with Dimethoate CL 12880 (Lot #611A). Since the submitted results are inconclusive, the study is considered to be unacceptable in the present form.

Study:

Micronucleus Test with Dimethoate CL 12880 (Lot #611A) Pharmakon Research International, Inc. Study No. PH 309 A-AC-004-84, March 7, 1985, Accession No. 257603.

Procedure:

1. The test compound, Dimethoate CL 12880, Lot #611A, was weighed, diluted with 0.9 percent saline, and administered by intraperitoneal injection to groups of five male and five female mice (Charles River CD-1 strain; 9-week old, body weight range 24 to 42 grams) at a single dose of 55 mg/kg and also, at the same dose of test compound on two occasions, 24 hours apart. Concurrently, five male and five female mice were used for the negative control (0.9% saline) and 5 male and 5 female mice were also used for the positive control (0.5 mg/kg Triethylenemelamine). At the end of specific time intervals, animals were sacrificed and femurs of each animal were removed according to the following post-treatment sampling times:

		Sampling Times (No. of Animals)			
		6 hrs	30 hrs	48 hrs	72 hrs
Treatment	Compound	M & F	M & F	M & F	M & F
Single Dose	Negative Control (0.9% Saline, 10 ml/kg)	-	10	-	-
	Positive Control (TEM, 0.5 mg/kg)	-	-	10	-
	Dimethoate, 55 mg/kg	10	10	10	10
Double Dose	Negative Control (0.9% Saline, 2 X 10 ml/kg)	-	-	10	-
	Positive Control (TEM, 2 X 0.5 mg/kg)	-	-	10	-
	Dimethoate, 2 X 55 mg/kg		10	10	10
	Total Number of Animals	10	30	50	20

2. Bone marrow cells were then flushed from femurs into 1 ml of fetal bovine serum in a conical centrifuge tube. Following centrifugation to pellet the cells, a drop of cell suspension was spread on a frosted glass microscope slide. The slides were air-dried and stained by Giemsa (pH 6.8).

3. One thousand polychromatic erythrocytes (PCE) were scored from each mouse for the presence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes was also calculated. The frequency of the cells containing micronuclei was determined. All single dose groups were compared pairwise to the single dose

negative control group and all multiple dose groups were compared to the multiple dose negative control group by using pairwise "t" test. Statistical significance was judged at $P < 0.05$ and $P < 0.01$ levels.

4. Dimethoate (O,O-dimethyl-S-(N-methyl carbamoylmethyl) phosphorodithioate is a crystalline, grayish white solid (97.3% purity).

Results:

Summary of Group Mean Erythrocytes

Treatment	No. of PCE Examined	No. of Cells*** W/Micronuclei	"t" Value	PCE/NCE Ratio	"t" Value
Single Dose					
Negative Control (0.9% Saline)	10000	0.5 ± 0.71	-	1.46 ± 0.41	-
Positive Control (0.5 mg/kg, TEM)	10000	64.4 ± 26.78	7.54**	0.91 ± 0.99	3.34*
Dimethoate (55 mg/kg)	6 hrs 10000	0.9 ± 1.10	0.97	1.50 ± 0.62	0.06
	30 hrs 10000	0.3 ± 0.48	0.74	1.69 ± 0.49	1.11
	48 hrs 10000	0.1 ± 0.32	1.63	1.44 ± 0.52	0.17
	72 hrs 10000	0.3 ± 0.67	0.65	1.98 ± 0.80	1.61
Multiple Dose					
Negative Control (0.9% Saline)	10000	0.8 ± 0.63	-	1.88 ± 0.41	-
Positive Control (2 X 0.5 mg/kg, TEM)	10000	63.2 ± 17.03	11.58**	0.88 ± 0.58	4.62**
Dimethoate (2 X 55 mg/kg)	30 hrs 10000	0.7 ± 0.67	0.34	1.47 ± 0.54	2.22*
	48 hrs 10000	0.2 ± 0.42	2.50	1.10 ± 0.44	4.66**
	72 hrs 10000	0.7 ± 0.67	0.34	1.48 ± 0.44	2.23*

*, ** Denotes statistical significance at $P < 0.05$ and $P < 0.01$ levels, respectively.

*** No. of cells with micronuclei per 1000 cells.

Findings:

1. The spontaneous rates of micronuclei in the polychromatic erythrocytes found from the single dose and multiple dose negative controls were within the normal range (e.g., 0.05 to 0.08%).

2. The highest concentration of the test compound (55 mg/kg) used in this study was selected from the preliminary dose-finding study. At the dose levels at 35 and 65 mg/kg of body weight only decreased activity and piloerection were observed in the treated animals. The abnormal clinical signs observed at 65, 95, and 125 mg/kg of body weight were more severe [redacted] and included tremors, ataxia and lacrimation. After 72 hours of initial dose administration, all of the animals also died at the highest two dose levels (95 and 125 mg/kg) while on study.

3. The positive control compound, TEM, apparently induced marked increase of the PCE with micronuclei which indicated the sensitivity of the assay ($t = 7.54$ to 11.58; $P < 0.01$). A significant depression of the bone marrow was also observed in these animals treated with TEM (e.g., significant decrease in the PCE/NCE ratio when compared with that of control).

4. The test compound did not induce any significant increase in the number of PCE containing micronuclei from animals treated with single or multiple doses of 55 mg/kg at all of the time intervals evaluated. But significantly depressed PCE/NCE ratios were noted in double-dosed dimethoate-treated animals.

Evaluation:

According to the acceptable procedure of mouse micronucleus assay (EPA Health Effects Test Guideline EPA 560/6-82-001), at least three dose levels of the test compound should be used for performing the mouse micronucleus test. Since the submitted results are based on the single dose of 55 mg/kg only, the study is considered to be inconclusive and unacceptable in the present form.