



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

004848

OFFICE OF,
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mutagenicity Studies with Chlorpyrifos
EPA ID No. 464-404; CASWELL #219AA; Tox. PN #804;

TO: Jay Ellenberger (12)
Registration Division (TS-767)

FROM: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

THRU: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769)
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-679)

DSB 12-10-85

Quang Bui for LOC
12/11/85

Action Requested

Review the mouse micronucleus and CHO/HGPRT gene mutation studies conducted with chlorpyrifos.

Recommendations

- 1) The mouse micronucleus test (study #TXT:K-044793-067) was negative for clastogenic effects. The study was classified as Acceptable.
- 2) The CHO/HGPRT gene mutation assay (study #HET K-044793-072) was negative for mutagenic effects. The study was classified as Acceptable.

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Data Evaluation RecordStudy Type: Micronucleus test in mice.Study Identification: "Evaluation of Chlorpyrifos in the Mouse Bone Marrow Micronucleus Test"Lab. performing study: Health and Environmental Sciences- Texas
Lake Jackson Research Center
Dow Chemical Co.

Freeport, Texas 77541

Sponsor:

Dow Chemical USA.

Midland, Michigan 48640

Study no.:

TXT:K-044793-067

Accession no.:

259603

Report date:

August, 1985

Submitted to EPA:

9/06/85

Study authors:

Gollapudi, B.B., Linscombe, V.A., and Wilkerson, J.E.

Reviewed By:

D. Stephen Saunders Jr., Ph.D.

Toxicologist, Section V

TOX/HED (TS-769)

DSJ (2-10-85)

Approved By:

Irving Mauer, Ph.D.

Geneticist, Toxicology Branch

Hazard Evaluation Division (TS-769).

J. J. Mauer
12-10-85

Conclusions: No effect of treatment on the incidence of micronucleus formation was apparent. A slight decrease in the ratio of PCEs to NCEs was noted in females at 24 or 48 hours after treatment ($p < 0.05$ in the high dose group), suggesting that the test article reached the bone marrow in sufficient concentration to produce an effect.

Classification: AcceptableMaterials and Methods

A. Materials- (1) Test chemical: Chlorpyrifos (0,0-diethyl-O-[3,5,6-tri-chloro-2-pyridyl]phosphorothioate), AGR214637, lot no. MM820905-610; 95.7% a.i. Positive control: cyclophosphamide, purchased from Sigma Chemical Co.

(2) Doses tested: Single doses of 0, 7, 22 or 70 mg/kg of test material by gavage in a volume of 6.0 ml corn oil/kg body weight.
Cyclophosphamide- 120 mg/kg by gavage in a volume of 12 ml corn oil/kg body weight.

(3) Test animal: Male and female CD-1 (ICR) BR mice, 5/sex/dose, obtained from Charles River, Kingston, NY.

Materials and Methods (con't)

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed and the following point(s) were noted:

1) Doses were selected on the basis of a range-finding study, in which an LD₅₀ value of 111 mg/kg was estimated by the testing facility for this strain of animal. The highest dose tested in the present study of 70 mg/kg was about 60% of the estimated LD₅₀.

Results

I. Range-Finding Study: Five mice of each sex were dosed with 0, 50, 100, 150 or 200 mg/kg of the test substance by gavage, and observed for 14 days. All mice survived a dose of 50 mg/kg for 14 days, however 1/5 males and 2/5 females died after a dose of 100 mg/kg within the 14-day observation period. Four of five males or females died after a dose of 150 mg/kg, and 5/5 males or females died after a dose of 200 mg/kg.

II. Micronucleus Study: A. Clinical Signs and Mortality- No toxic signs were reported for any of the test animals. A single control (corn oil) female died within 24 hours of treatment, and was not examined for micronuclei. No other deaths were noted.

B. Micronucleus Incidence- No effect of treatment with chlorpyrifos on the incidence of micronuclei/1000 polychromatic erythrocytes (PCE) was apparent in either males or females at 24 or 48 hours after treatment (Tables 2 and 3, photocopied from the study report). No effect of treatment on the ratio of PCEs to NCEs (normochromatic erythrocytes) was apparent in males, however a slight, apparently dose-related, decrease in this ratio was observed in treated females. The %PCEs was significantly different from control ($p < 0.05$ by Dunnett's T-test, calculated by Dr. H. Lacayo of the Tox. Branch Statistical Support Team) in the female high dose (70 mg/kg) group at 24 or 48 hours after treatment.

The positive control, cyclophosphamide, induced the expected large increase in the ratio of micronuclei to PCEs in both sexes within 24 hours of treatment, demonstrating that the test system was sensitive to the effects of a known mutagen. These data are presented in Tables 2 and 3, photocopied from the study report.

Conclusion

No effect of treatment with chlorpyrifos on the incidence of micronuclei was apparent. Treatment with the test chemical induced an apparent dose-related, statistically significant, decrease in the ratio of PCEs to NCEs in the high dose female treatment groups, suggesting that the test article reached the bone marrow and produced a minimally toxic effect there. A much larger decrease in this ratio was noted with the positive control. Therefore, it is concluded that the test chemical has no clastogenic potential in the bone marrow of the mouse.

Classification: Acceptable

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Data Evaluation Record

004849

Study Type: Gene mutation assay in mammalian cells (in vitro).

Study Identification: "Evaluation of Chlorpyrifos in the Chinese Hamster Ovary Cell-Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay."

Lab. performing study: Health and Environmental Sciences
Mammalian & Environmental Toxicology Research Lab.
Dow Chemical USA
Midland, Michigan 48674

Sponsor:

Dow Chemical USA
Midland, Michigan 48674

Study no.:

HET K-044793-072

Accession no.:

259603

Report date:

September 3, 1985

Submitted to EPA:

9/06/85

Study authors:

Mendrala, A.L. and Schumann, A.M.

Reviewed By:

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DSD
12-10-85

Approved By:

Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

12-10-85

Conclusions: Negative for gene mutations, with or without metabolic activation.

Classification: Acceptable

Materials and Methods

A. Materials- (1) Test chemical: Chlorpyrifos [0,0-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate], lot no. MM 820905-010; 95.7% a.i. Positive control with metabolic activation: 3-methylcholanthrene (3-MC, 5 ug/ml, 18.6 uM) dissolved in DMSO; without metabolic activation: ethyl methanesulfonate (EMS, 370 ug/ml, 3 mM) dissolved in DMSO.

(2) Concentrations tested: 0, 10, 20, 25, 30, 40, and 50 uM of chlorpyrifos dissolved in DMSO with and without metabolic activation (S-9); the final concentration of DMSO in all test cultures was 1%.

(3) Test species: Chinese hamster ovary cells, subclone CHO-k₁-BH₄, obtained from Dr. Abraham Hsie, Oak Ridge National Lab., Oak Ridge, TN.

Materials and Methods (con't).

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed and the following point(s) were noted:

1) Doses were selected on the basis of a preliminary cytotoxicity study. Concentrations of chlorpyrifos of greater than 30 μ M were reported to form a precipitate in the test system, with or without metabolic activation.

2) At the higher test concentrations, when lower survival was expected, the number of cells seeded was increased from 200 to 1000 per dish.

Results/Discussion

Data were submitted as individual findings with calculated means, (appended to this review as Tables I and II, photocopied from the study report).

In the absence of metabolic activation, no effect of treatment on the incidence of formation of thioguanine-resistant mutants was noted (Table I). The positive control, EMS, produced the expected large increase in the number of mutants, demonstrating the sensitivity of the test system to a direct-acting mutagen. Concentrations of chlorpyrifos greater than 10 μ M were cytotoxic as evidenced by decreases in cloning efficiency and relative survival, and concentrations of chlorpyrifos of 30 μ M and greater were reported to form a precipitate.

Similarly, no effect of treatment on the frequency of mutants was noted in the presence of metabolic activation (S-9 fraction of rat liver homogenate). In contrast to the non-activation assay, no effect on relative survival or cloning efficiency was apparent, suggesting a detoxication effect of the added liver enzymes. However, a precipitate was reported for concentrations of chlorpyrifos of 30 μ M and greater. The positive control, 3-MC, produced the expected large increase in the number of mutants, demonstrating the sensitivity of the test chemical to a mutagen that requires metabolic activation.

Classification: Acceptable

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