8-27-86

AUG 27 1986

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MEMORANDUM

SUBJECT: Mutagenicity Studies With Chlorpyrifos.
EPA Reg. No. 464-404; Tox. PN #1354; Caswell #219AA

TO: Larry Schnaubelt (12)
Registration Division (TS-767C)

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

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

Action Requested

Review the submitted rat hepatocyte DNA repair study and the Salmonella gene mutation assay.

Recommendations

1. The rat hepatocyte DNA repair assay (study #HET K-044793-073) was negative for genotoxic effects, and was classified as Acceptable.

2. The Salmonella/microsome gene mutation assay (study #TXT:K-044793-075) was negative for gene mutations, and was provisionally classified as Acceptable. As only summary data were submitted, the Registrant is requested to submit individual culture findings from this study (similar to those submitted for the rat UDS study, #HET K-044793-073) in order to make this study fully acceptable.

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DATA EVALUATION RECORD 005424

STUDY TYPE: Rat hepatocyte unscheduled DNA synthesis assay. (Guidelines 84-2)

ACCESSION NUMBER: 261821 MRID NUMBER: 157057

TEST MATERIAL: Chlorpyrifos (Caswell #219AA)

REPORT NUMBER: HET K-044793-073

SPONSOR: Dow Chemical USA
Midland, MI 48640

TESTING FACILITY: Dow Chemical
Health and Environmental Services
Mammalian and Environmental Toxicology
Research Lab.
Midland, MI 48674

TITLE OF REPORT: "Evaluation of Chlorpyrifos in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay."

AUTHORS: Mendrala, A.L. and Dryzga, M.D.

REPORT ISSUED: 1-31-86

DSS 8/21/86

Reviewer: D. Stephen Saunders, Ph.D.
Toxicologist, Section 7, Toxicology Branch

Secondary Reviewer: Irving Mauer, Ph.D.
Senior Geneticist, Toxicology Branch

Conclusion: Chlorpyrifos was negative for UDS in isolated rat hepatocytes under the conditions of this study.

Classification: Acceptable

Materials and Methods

A. Materials: (1) Test material- Chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl]phosphorothioate), reference AGR 214637, MM 820905-610; 95.7% a.i.

(2) Test Species: A single male Fischer 344 rat, obtained from Charles River Breeding Labs., Wilmington, MA. This animal was 12 weeks of age and weighed 248 grams at study initiation.

B. Study Design

Rat hepatocytes were isolated and cultured using standard techniques (Williams, G.M., Cancer Res. 37:1845-1851, 1977; Williams, G. M. et al., In Vitro 13:809-817, 1977). Briefly, the rat was anesthetized, the liver was exposed surgically, and the hepatic portal vein was cannulated. The rat was administered heparin, and then perfused with EGTA and collagenase. The liver was removed, and cells were removed and sedimented be mild centrifugation, and then resuspended in incubation medium. Cell viability, reported to be 86%, was determined by trypan blue exclusion.

Approximately 5 X 10^5 cells were placed in culture dish containing a plastic coverslip and 2 ml of incubation medium. Cells were then incubated under standard conditions for 1.5 hours to allow attachment of cells to the coverslip.

Molar concentrations of chlorpyrifos of 1 x 10^{-4}, 3.16 x 10^{-5}, 1 x 10^{-5}, 3.16 x 10^{-6} and 1 x 10^{-6} M in 0.1% DMSO or 1.0% DMSO (high dose only) were added to test cultures, and cells were incubated for 18 hours with medium containing 10 uCi/ml ^{3}H-thymidine under standard conditions. The positive control was 2-acetylaminofluorene (2-AAF), added in concentrations of 10^{-7} to 10^{-5} M. Cells were then washed with cold thymidine, fixed, dried, and mounted on glass slides.

Slides were dipped in photographic emulsion, and then stored for 10 days, after which time grains were developed (Kodak D-19). Slides were counter-stained with hematoxylin-eosin. The net grain count (indicating UDS) was determined for 15 cells on each of two slides for each dose level by counting (using an automatic grain counter) the number of grains in wach nucleus and subtracting the mean number of grains contained in 3 adjacent nucleus-sized areas. Cells undergoing active DNA replication (indicated by completely blackened nuclei) were not included in the analysis.

The test was considered positive if the number of net nuclear grains was greater than 6/nucleus, and was significantly different from control ($p<0.05$) as determined by the Kruskal-Wallis ANOVA and Wilcoxin's Rank Sum test with Bonferoni's correction.

Results/Discussion

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Incubation of cells with the two highest concentrations of chlorpyrifos (3.16×10^{-5} or 1×10^{-4} M) was found to be cytotoxic, as defined by detachment of cells from the coverslip and/or a "granular" appearance. In addition, a precipitate was observed at the highest concentration, 10^{-4} M. Therefore, a sufficient range of concentrations was tested.

No significant effect of treatment on the incidence of UDS was apparent (Table II, photocopied from the study report). The positive control, 2-AAF, induced the expected large increase in the net number of nuclear grains, demonstrating the sensitivity of the test system.

Classification: Acceptable

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DATA EVALUATION RECORD

STUDY TYPE: Gene mutations in the Salmonella/microsome assay. (Guidelines 84-2)

ACCESSION NUMBER: 261821 MRID NUMBER: 157058

TEST MATERIAL: Chlorpyrifos (Caswell #219AA)

REPORT NUMBER: TXT:K-044793-075

SPONSOR: Dow Chemical USA
Midland, MI 48640

TESTING FACILITY: Dow Chemical Company
Health and Environmental Sciences-Texas
Lake Jackson Research Center
Freeport, TX 77541

TITLE OF REPORT: "Chlorpyrifos: Evaluation in the Salmonella/ Mammalian Microsome Mutagenicity Assay."

AUTHORS: Bruce, R.J. and Zempel, J.A.

REPORT ISSUED: February, 1986

Reviewer: D. Stephen Saunders, Ph.D. DSS 8/21/86
Toxicologist, Section V, Toxicology Branch

Secondary Reviewer: Irving Mauer, Ph.D.
Senior Geneticist, Toxicology Branch

Conclusion: Chlorpyrifos was negative for gene mutations in Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538, with or without metabolic activation under the conditions of this study. As only summary data were submitted, the classification is considered provisional, pending submission of individual culture findings.

Classification: Acceptable (Provisional, pending submission of individual findings).

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Materials and Methods

A. Materials: (1) Test material- Chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl]phosphorothioate), reference AGR 214637, lot MM 820905-610; 95.7% a.i. Positive Controls- N-methyl-N'-nitro-N-nitrosoguanine 2 ug/plate (non-activated, TA100 and TA1535); 2-nitrofluorene 100 ug/plate (non-activated, TA98 and TA1535); quinacrine mustard 10 ug/plate (non-activated, TA1537); 2-anthramine 5 ug/plate (activated, TA100 and TA1535); 2-acetylaminofluorene 100 ug/plate (activated, TA98 and TA1538); 3-aminoquinoline 25 ug/plate. All chemicals were "of reagent quality or better".

(2) Test Species: Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538, obtained from Dr. Bruce Ames, U. Calif.-Berkeley.

B. Study Design

Bacterial tester strains were grown under standard culture conditions. Rat liver S-9 fraction, derived from Aroclor 1254 induced male Sprague-Dawley rats, was obtained from Sitek Research Laboratories.

Bacteria, test chemical, and required buffers and cofactors were preincubated with or without metabolic activation for 30 minutes at 30°C with gentle agitation. Chlorpyrifos was tested at concentrations of 1, 3.16, 10, 31.6 and 100 ug/plate. All test concentrations and positive and negative controls were assayed in triplicate cultures. After the 30 minute preincubation, 2 ml of agar were added to each sample, and the mixtures were poured onto plates and incubated at 37°C for 48 hours. At the end of the 48 hour incubation period, revertant colonies were counted using an automatic colony counter (Artek Systems). The investigators considered a response as positive "if the mean number of revertant colonies is at least three times higher then the mean of the negative control and it produces a dose response relationship over several concentrations". Increased reversion frequency in the absence of a dose-effect relationship was considered "presumptive" evidence of mutagenicity, whereas an increase in reversion frequency of >2x but <3x (relative to control) was considered to be inconclusive.

Results/Discussion

None of the tested concentrations of chlorpyrifos, including the highest concentration, 100 ug/plate, which formed a precipitate and was also reported as toxic (reduced growth), produced any evidence of mutagenicity in any of the tester strains (Table III, photocopied from the study report). Metabolic activation had no effect on this negative response. As the test material was tested up to the limits of cytotoxic-

ity and solubility, a sufficient range of concentrations was tested. The positive controls produced the expected increases in the number revertants, thereby demonstrating the sensitivity of the test system.

As only summary data were submitted, individual culture findings are required before this study will be fully acceptable.

Classification: Acceptable (provisional, pending submission of individual findings).

TABLE III

Pre-incubation Assay
Mean* Revertant Colonies Per Plate

Test Chemical: Chlorpyrifos

Test Concentration (µg/plate)	TA98		TA100		TA1535		TA1537		TA 1538	
	NA	A	NA	A	NA	A	NA	A	NA	A
100.0	14±4P	21±5P	91±14TP	117±8TP	8±4TP	5±1TP	4±3TP	2±4TP	7±2TP	12±6TP
31.62	14±5	21±2	88±21	95±6	9±4	7±2	3±2	8±2	8±1	15±2
10.0	12±3	20±5	95±18	118±24	3±2	6±1	6±2	4±1	11±4	13±4
3.162	13±1	20±6	96±9	105±10	2±1	9±4	6±1	8±6	7±3	16±3
1.0	11±2	18±6	78±3	96±6	3±2	6±4	3±1	6±4	9±3	13±7
0[a]	12±1	24±8	97±8	104±10	9±4	10±6	6±3	6±2	11±3	10±6
							4±1 (H_2O)			
Spontaneous Reversion	15±2	19±5	104±8	136±6	8±3	9±4	7±1	7±4	12±3	12±2
Positive Control	1864±69	2119±123	1158±41	1239±108	955±19	169±30	206±5	640±61	2372±285	2327±78

*=Mean and S.D. of triplicate plates.
NA=Nonactivated.
A=Activated.
a=Solvent control is DMSO Unless designated otherwise.
T=Toxicity (poor background lawn and/or overgrown background colonies).
P=Test material formed a precipitate in the medium.