10-1-85

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Supplement to the Mouse Oncogenicity Study With Chlorpyrifos.
EPA ID. No. 464-404 CASWELL #219AA

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

FROM: D. Stephen Saunders Jr., Ph.D. DSS 9/30/85
Toxicologist, Section V
TOX/HED (TS-769)

THRU: Laurence D. Chitlik, DABT LDC 10/1/85
Head, Section V
TOX/HED (TS-769)
and
Theodore M. Farber, Ph.D. 10/29/85
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

Action Requested

Review the supplement to the chlorpyrifos mouse oncogenicity study, submitted in response to the chlorpyrifos registration standard.

Recommendations

Toxicology Branch recommends that the mouse oncogenicity study (MRID #00054352) be upgraded to Core-Minimum status.

Discussion

The submitted addendum was a four-week dietary feeding study in CD-1 mice, which tested the effect of doses of 0 and 15 ppm of chlorpyrifos. This study was submitted in response to the chlorpyrifos registration standard, and was intended to demonstrate that 15 ppm, the highest dose tested in the mouse oncogenicity study (MRID #00054352), was adequate.

These data demonstrate that 15 ppm of chlorpyrifos in the diet of CD-1 mice is a maximally tolerated dose (MTD). A 25% decrease in weight gain was noted in males after four weeks of treatment, and approximately 90% and 50% inhibition of plasma and erythrocyte cholinesterase activities, respectively, were noted in males and females after one or four weeks of treatment. The study is classified as Core-Minimum data when considered with the results of the mouse oncogenicity study.

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Background

These data were submitted in response to the chlorpyrifos registration standard, in which the mouse oncogenicity study was re-examined by Mr. W. Burnam who concluded that there was no evidence that the highest dose tested was a Maximally Tolerated Dose (MTD). This study was originally reviewed by Dr. W. Dykstra, and has also been reviewed by Mr. R. Jaeger for the World Health Organization (see attached photocopy). Dr. Dykstra and Mr. Jaeger both concluded that chlorpyrifos was not oncogenic in the mouse. This reviewer has re-examined the summary tumor tables from the original study, and agrees with the conclusions of Dr. Dykstra and Mr. Jaeger that no oncogenic potential was demonstrated in this study.

Additionally, the MTD question was addressed by Mr. Jaeger in his review. Mr. Jaeger indepehdently concluded that the highest dose tested of 15 ppm was sufficient since a mouse teratology study, in which similar doses of chlorpyrifos were tested, demonstrated significant depression of plasma and erythrocyte cholinesterase activities. Therefore, Mr. Jaeger concluded "that 15 ppm of chlorpyrifos in the diet of mice for 105 weeks is not without demonstrated toxicity in the laboratory animal."

Data Evaluation Record

004747

Study Type: Addendum to the mouse oncogenicity study.

Study Identification: "Chlorpyrifos: A Four-Week Dietary Study in CD-1 Mice".

Lab. performing study:	Mammalian and Environmental Toxicology Research Labs. Midland, MI 48640 Dow Chemical USA
Sponsor:	Dow Chemical USA
Study no.:	HET K-044793-068
Accession no.:	073608
Report date:	5/23/85
Submitted to EPA:	6/04/85
Study authors:	Davies, D.B., Tollett, J.T., and Lomax, L.G.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769)
Approved By:	Laurence D. Chitlik, DABT Head, Section V TOX/HED (TS-769)

Conclusions: Significant decreases in body weight gain of 25% were noted in treated males at four weeks, and decreases of 90% and 50% in plasma and RBC cholinesterase activities, respectively, were noted in males and females after one or four weeks of exposure to diets containing 15 ppm chlorpyrifos, the only dose tested. These data demonstrate that 15 ppm, the highest dose tested in the mouse oncogenicity study (MRID #00054352), was adequate, and approximated a maximally tolerated dose (MTD).

Classification: Core-Minimum When considered with the data from the mouse oncogenicity study.

Background

This addendum was submitted in response to the chlorpyrifos registration standard (memo G. Burin to J. Ellenberger, 5-25-84), in which the mouse oncogenicity study (MRID #00054352) was re-examined by W. Burnam. That study was found deficient because there were insufficient data to demonstrate that the highest dose tested (15 ppm) was a Maximally Tolerated Dose (MTD).

The present submission is a 4-week dietary feeding study conducted in the same strain of mice (CD-1) as the original oncogenicity study. A control and high dose (15 ppm) group were tested, where the high dose was the same as in the oncogenicity study. Parameters such as plasma, erythrocyte, and brain cholinesterase (ChE) activities were monitored so as to demonstrate that the high dose in the original oncogenicity study was sufficiently high as to be "considered representative of the maximum-tolerated dose for this strain of mouse".

004747

Materials and Methods

A. Materials: (1) Test Compound- Dursban F insecticide; chlorpyrifos; reference #AGR 214637, lot #MH820905-610; 95.7% a.i.; supplied by the Agricultural Products Dept., Dow Chemical Co.

(2) Doses tested- 0 and 15 ppm in the feed, for one or four weeks.

(3) Test animal- Male and female CD-1 mice, 20/sex/dose, supplied by Charles River Breeding Laboratories, Portage, MI.

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

(1) Only a control and high dose group were studied.

(2) Data for physical examinations were not submitted.

(3) No histological examinations of tissues were reported.

Results

A. Test Diet Analyses: Samples of the original and final diet preparations demonstrated that test diets were within 7% of targeted levels. Data submitted from a previous study demonstrated that chlorpyrifos remained stable in animal feed, kept at room temperature, for up to 56 days.

B. Body Weights and Food Consumption: Data were submitted as summary and individual animal body data, determined at weekly intervals.

The test article caused a statistically significant decrease of about 25% in body weight gain ($p < 0.05$) in treated males after four weeks of treatment, but had little effect on weight gain in females (Table 1). In the calculation of body weights after one week of treatment, the Registrant separated the body weights of animals sacrificed at one week from the weights (at one week) of those animals remaining on test for four weeks. Since all animals were placed on test at the same time, there is no statistically valid reason for separating the body weights of interim sacrifice animals from the remaining animals designated to be treated for four weeks. Although a statistically significant difference was noted at one week between the body weights of control and treated males sacrificed after four weeks of treatment, when the body weights of mice sacrificed at one week were included, no difference was apparent.

Since food consumption was similar in control and treated males at each of the weekly intervals (data not shown) the apparent decrease in body weight gain observed at week 4 was likely a compound-related effect, and could not be attributed to rejection of food by treated animals. Similarly, no effect of treatment on food consumption in females was apparent.

Table 1. Body Weights and Food Consumption[a]

Week	Males		Females	
	0 ppm	15 ppm	0 ppm	15 ppm
0[b]	28.6 ± 1.8	28.5 ± 1.9	22.9 ± 1.4	23.1 ± 1.4
0[c]	28.8 ± 1.8	28.8 ± 1.8	23.3 ± 1.4	23.2 ± 1.4
1[b]	30.7 ± 1.8	31.0 ± 1.5	24.7 ± 1.7	24.8 ± 1.5
gain	2.1 ± 0.9	2.5 ± 1.1	1.8 ± 0.6	1.7 ± 0.9
1[c]	31.1 ± 1.8	30.9 ± 1.7	25.2 ± 1.7	24.9 ± 1.4
gain	2.3 ± 0.9	2.2 ± 1.1	1.9 ± 0.7	1.7 ± 0.8
2	32.8 ± 1.7	32.2 ± 2.2	26.1 ± 1.9	25.2 ± 1.6
gain	3.7 ± 1.3	3.2 ± 1.5	2.3 ± 1.3	1.9 ± 1.1
3	33.8 ± 1.8	32.8 ± 2.1	27.0 ± 1.7	26.4 ± 1.6
gain	4.8 ± 1.7	3.8 ± 1.8	3.2 ± 1.2	3.1 ± 1.3
4	34.6 ± 2.0	33.3 ± 2.1*	27.5 ± 1.5	27.1 ± 2.0
gain	5.6 ± 1.9	4.2 ± 1.9*	3.8 ± 0.9	3.8 ± 1.7

[a]data excerpted from submitted study. Values are in grams, mean ± standard deviation calculated by the investigators.
[b]mice sacrificed after one week of treatment only.
[c]data for all animals on test.
*$p < 0.05$ by Dunnett's tests.

C. Clincal Signs: Actual data for physical observations were not submitted. The investigators stated that "No significant or unusual in-life clinical observations were noted during the study. No clinical symptoms indicative of organophosphate intoxication were evident among treated animals of either sex. All mice survived until their pre-designated sacrifice."

D. Cholinesterase Activities: Data were submitted as summary and individual animal values.

Statistically significant reductions in plasma and erythrocyte cholinesterase (ChE) activities were noted in treated males and females after one or four weeks of treatment (Table 2). After one week, plasma and red cell ChE activities were inhibited by about 90% and 50%, respectively, relative to control values. Similar decreases were noted at the four-week sacrifice. No significant difference between males and females in the degree of inhibition was apparent. Brain ChE was not affected after one week of treatment, however after four weeks of treatment a statistically significant decrease in males of about 6%, and a statistically significant increase in females of about 5%, was noted. The investigators stated that this apparent effect was of "questionable toxicological significance" in light of the small degree of change and lack of consistent effect between males and females. This reviewer agrees with that assessment. Since a similar decrease in plasma and RBC ChE was noted in both males and females, a true treatment-related decrease in brain ChE would be expected to produce similar effects in both sexes.

Table 2. Cholinesterase Activities[a]

	Males		Females	
	0 ppm	15 ppm	0 ppm	15 ppm
One Week Sacrifice				
Plasma (% control)	53.7 ± 9.3	6.3 ± 1.6** (11.7)	87.5 ± 9.1	7.7 ± 1.6** (8.8)
Erythrocyte (% control)	9.3 ± 1.0	4.4 ± 0.5** (47.3)	7.4 ± 0.8	4.4 ± 0.5* (59.5)
Brain (% control)	132.8 ± 3.9	134.7 ± 12.0 (101)	142.2 ± 9.4	139.7 ± 12.7 (98.2)
Four Week Sacrifice				
Plasma (% control)	59.5 ± 12.6	5.2 ± 1.1** (8.7)	94.0 ± 14.5	8.5 ± 1.9** (9.0)
Erythrocyte (% control)	9.1 ± 1.2	4.3 ± 0.8* (47.3)	8.1 ± 0.8	3.8 ± 0.5* (46.9)
Brain (% control)	140.2 ± 7.1	131.7 ± 6.0* (93.9)	120.9 ± 7.4	126.9 ± 6.4* (105)

[a]data excerpted from submitted study. Values are in tenths of an international unit/ml, mean ± standard deviation calculated by the investigators.
*$p < 0.05$ by Dunnett's test.
**$p < 0.05$ by Wilcoxon's test.

E. Necropsy Data: (1) Organ weights- Data were submitted as summary and as individual animal values.

No toxicologically significant effect of treatment on absolute or relative organ weights was apparent. Absolute organ weights of brain, heart, kidneys, liver, and testes (the only tissues weighed) tended to be about 10% less in treated males sacrificed at four weeks than in control mice, however none of these changes was judged to be statistically significant. Since relative organ weights in these animals were not affected to a similar extent, the apparent effects on absolute organ weights may have been related to the decrease in weight gain noted for treated males.

A statistically significant decrease in absolute brain weight of about 4% was noted in treated males sacrificed at one week, however this apparent effect is of doubtful toxicological significance since a similar statistically significant effect was not apparent at 4 weeks, and relative brain weights at one week were not different from control.

E. (2) Gross observations- Data were submitted as a summary table and as individual animal observations for mice sacrificed at four weeks.

No treatment related effects on gross pathology were apparent. Findings noted without apparent relation to treatment included abcessed seminal vesicle (1/20 control males), abcessed coagulating gland (1/20 control males), cystic ovary (1/20 control females), distended uterus (2/20 control females), and bilateral external ear canal scabs (1/20 control males). All other tissues were judged to have "no visible lesions".

Discussion

This four-week feeding study in mice was intended to demonstrate that the highest dose tested of 15 ppm in the mouse oncogenicity study was adequate, i.e. was an MTD. The effects observed in the present study included a decrease in body weight gain over four weeks of about 25% in treated males (but not females), with no effect on food consumption. Therefore, a decrease in food efficiency as a result of treatment was apparent. Decreases of about 90% and 50% in plasma and erythrocyte cholinesterase activities after one or four weeks of treatment were also noted in males and females. Other parameters such as brain cholinesterase, organ weights, and gross pathological observations were not affected in a toxicologically significant manner by treatment with the test article.

These data demonstrate that chlorpyrifos produced toxic effects, in the form of decreased body weight gain and cholinesterase activities, in treated mice. The significant reduction in cholinesterase activities after four weeks of treatment suggests that the concentration of test article in the diet could not have been increased significantly without potential induction of cholinergic symptoms over the course of a two-year chronic feeding study.

Classification: Core-Minimum When considered with the data from the mouse oncogenicity study (MRID #00054352).