12-22-87

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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Rev. Sec. # 1/Toxicology Branch
Hazard Evalauation Division TS-769C

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FROM: D. Ritter, Toxicologist
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Hazard Evalauation Division TS-769C

12/22/87

Caswell #: 374.
Tox. Proj. # : 7-1055
Registrant: Mobay Corporation, Kansas City, MO.

Subject: EPA ID # 3125-108 - Azinphos Methyl; Guthion Technical; R-1582.

1. Review of Two Generation Rat Study submitted under § 6(a)(2).

Our review of the subject study is attached. The Maternal NOEL is 15 ppm. The Reproduction NOEL is 5 ppm. The study is rated CORE Minimum.

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2. Review of a 28 day cholinesterase study and a chronic toxicity study in virgin SPF Wistar female rats. Acc. # 403326-02; Study # 94817, 8/27/87, D. L. Van Goethem.

These studies are used to compare the toxic response noted in the 2-generation reproduction study reviewed above with the results of a 28 day range-finding feeding study and a chronic/oncogenicity study in which plasma , red cell and brain cholinesterase were reported for female rats. These studies are not acceptable for fulfilling data rquirements under EPA guidelines because no detailed analytical data are included and only the briefest outline of a protocol is provided. In addition there are no data for young and very young animals.

Virgin female rats were offered diets containing 0, 5, 20 or 50 ppm azinphos-methyl for 28 days. ChE levels were reported only for the 28 day period. Plasma ChE was significantly inhibited only at the 50 ppm level, being reduced to 60.5 % of the control dose level. Red cell inhibition was signicantly reduced at the 20 ppm and 50 ppm levels, being inhibited 27.4 % and 34.6 %, respectively. Brain ChE activity was significantly reduced only at the 50 ppm level, being depressed to 53.5 % of control value.

In the chronic/oncogenicity study, virgin female rats were offered diets containing 0, 4, 15 or 45 ppm azinphos-methyl for 12 months. Plasma, and red cell ChE were measured at 1, 3, 6 and 12 months. Brain ChE inhibition was measured at 12 months.

Plasma and red cell inhibition was reported to occur at the 15 and 45 ppm levels at all test periods. Brain ChE was significantly reduced only at the 45 ppm level.

The investigators did not report whether there were clinical or cageside signs of cholinesterase inhibition in either of these studies.

In attempting to link the findings of these studies with the 2 generation reproduction study, the investigators suggest that the F0 and F1B females, who would be approximately the same size and age, actually showed toxic signs that were related to increased ingestion of treated feed normally expected of gravid and lactating dams, and that the mortality of both the dams and the pups in this study were primarily due to an increased level of AI. However, they did not report an increase in feed consumption in these animals, except for the F1B - 45 ppm dams. [No attempt was made to measure ChE inhibition in these rats].

As we noted in our review of this study, the loss of pups could have been due either to a direct, transplacental exposure to AI, or it could have been due to reduced availability of maternal nourishment, or both.

It makes sense to consider that the increased toxic sequeleae reported in the 2 generation study could be due to increased maternal/offspring sensitivity to ingested AI. But these data do not of themselves support this contention.

Reviewed by: D. Ritter, Toxicologist DLR 12-16-87
Section I , Tox. Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head
Section I , Tox. Branch (TS-769C)

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DATA EVALUATION RECORD

STUDY TYPE: 2 Generation Reproduction, rat. TOX. CHEM. NO. 374

ACCESSION NUMBER: 403326-01 MRID NO.: NA

TEST MATERIAL: R-1582; EPA Guideline # 83-4.

SYNONYMS: Azinphos Methyl; Guthion

STUDY NUMBER(S): T6006415 (94824).

SPONSOR: MOBAY Corporation, Kansas City, MO.

TESTING FACILITY: Bayer AG, Fachbereich Toxikologie, FRG.

TITLE OF REPORT: Two Generation Study on Rats.

AUTHOR(S): R. Eiben & B. Jandra.

REPORT ISSUED: 3/10/87. (Study performed from September, 1982 to February, 1984).

CONCLUSIONS: R-1582 produces maternal toxicity at 45 ppm but not at doses of 5 and 15 ppm. The reproductive NOEL in this study is 5 ppm.

Classification: CORE - Minimum data. Only 5 breeders per sex were used to produce the 45 ppm F2A and F2B litters. Not repairable.

Special Review Criteria (40 CFR 154.7) None exceeded.

A. MATERIALS:

1. Test compound: R-1582. Description: Not stated.

   Batch #: 79/R255/42. Purity: 87.2%.

2. Test Species: Rat. Strain: BOR:WISW (Wistar Strain).

   Age: 5-6 weeks. Weight: 78-87 Gm. Source: Winkelmann, FRG.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned 12 M & 24 F to the following test groups:

Control	0.0	ppm
Low Dose	5.0	ppm
Mid Dose	15.0	ppm
High Dose	45.0	ppm

2. Compound Administration

Test Material was mixed into the feed in appropriate amounts each week.

Samples of treated feed were analyzed for stability at days 0, 1, 2, 7 & 10 following mixing with feed. Samples were tested for nominal concentration of Test Material in February, May, August and November of 1983.

3. Quality assurance procedures were acceptable and a certificate of Quality Assurance was provided.

4. Gravid animals were weighed initially, then weekly thereafter for 20 days following mating. All other animals were weighed weekly.

5. Animals received feed and water ad libitum.

6. Feed consumption and compound intake were determined weekly.

7. Husbandry - Standard GLP.

8. Animals were inspected daily for signs of toxicity and mortality.

9. Necropsy and Pathology:

All rats dying during the study or which were moribund were necropsied and subjected to gross examination. All parental animals were killed at 1 - 3 weeks after the last generation was weaned. The following organs and tissues were obtained and fixed in 10% buffered formalin:

Brain	Pituitary	Liver*
Kidneys*	Testes*	Epididymes
Sem. Ves.	Prostate	Ovaries*
Uterus	Vagina	Gross Lesions

* weighed for F1B parental rats.

10. Statistics - Population counts were tested by the "t" test of Mann, Whitney & Wilcoxon. The Reproductive Indices were calculated after Clopper & Pearson. We used Students "t" test for comparing weaned litter weights.

11. Mating & Reproduction

The initial breeding of the F0 and the F1B generation of rats was accomplished using 12 males and 24 females (2 females with 1 male). Males were interchanged so that each female was exposed to at least three males, unless mating occured first. The day of vaginal plug or positive sperm finding was day 0 of gestation. Mated females were placed in individual cages to cast and rear their litters. All pups were examined grossly at birth and during lactation for gross developmental anomalies. Pups were weighed at birth, culling and weekly thereafter. All pups were culled to eight per litter on day 5 of gestation. F1A and F2A generation pups were weaned at 4 weeks and then destroyed. F1B pups were selected for further mating such that the chance that littermates would breed together was minimized. F1B pups were reared to about 100 days of age, then mated to produce the F2A and F2B generations. Only five of these offspring per sex from the 45 ppm group were available for mating. This was due to high mortality of the dams and also of the pups in this group.

C. RESULTS:

F0 (Parental Generation).

a. Body Weights and Feed/Compound Ingestion

No significant differences in these parameters were noted for treated males at any dose level. No significant differences were reported for F0 treated females in these parameters for the 5 ppm or 15 ppm groups; the 45 ppm females failed to gain weight as well as the lower dose groups. Feed/compound intake was not affected by the Test Material at any dose level for either males or females.

b. Mortality and Signs of Toxicity

Mortality: 2 low-dose dams expired; one at birth and one during lactation. One middle-dose female died and one high-dose male and five females died, four of these during lactation. Signs of toxicity, confined to the 45 ppm group, included alopecia, poor coat condition, inflamed eyes, poor physical condition and convulsions.

c. Necropsy of these animals showed that the 45 ppm females had dark red blotches on the lungs. There were no histopathological findings that could be attributed to treatment, however. Organ to body weight ratios were not affected by exposure to Test Material in the F1B group.

d. The systemic NOEL in these animals is 15 ppm.

F1B (Parental Generation).

a. Body Weights and Feed/Compound Ingestion

Treated animals in the low and middle dose groups had body weight increases that were similar to those of the controls. 45 ppm males and females were significantly lighter than the controls. 45 ppm animals consumed significantly more feed than did the lower groups and the controls.

b. Mortality and Signs of Toxicity

One control dam and two 15 ppm dams died during suckling.

Some convulsions were reported in the 45 ppm group as was poor physical condition and unpreened coats. This group of dams also suffered severe mortality; at 5 days post-partum only 7 dams were alive and nursing 39 pups; at weaning (4 weeks) only 3 dams were alive nursing 18 pups. (These pups served as the breeders for the F2A and F2B litters).

c. Necropsy of these animals failed to reveal any lesions attributable to treatment.

d. The systemic NOEL is 15 ppm in these animals.

Litters

No developmental abnormalities were reported in any litter. All litters cast contained males and females in approximately the same ratios.

Table I represents the Sponsor's calculated Indices of Reproduction. The indices for fertility, gestation and live births indicate that the pre-natal phase of reproduction in this study was not materially affected by exposure to Test Material prior to birth of the pups. The Low-dose F2B litters suffered a slight but significant reduction in the indices for Viability and Lactation; the Middle-dose F1A and F2B groups were significantly reduced for viability and the F1B and F2A groups had reduced lactation indices. The High-dose groups, with the exception of the F2B litters, were most severely affected, suffering massive reductions in the viability

the fact that there were losses in both pups and dams that began to manifest themselves at the time of culling (5 days post-partum) to eight pups per litter. At weaning of the F1B pups. only only three litters were availble, containing 18 pups, to produce the F2A and F2B litters in this dose group We note that no attempt was made to place excess pups from one litter as "foster" pups in other litters lacking sufficient pups to produce full litters.

C. DISCUSSION AND CONCLUSIONS

From the reproductive indices summarized in Table I we note that dietary exposure to Test Material had no adverse effects on conception or gestation per se in the 5 and 15 ppm groups and only a slight effect on the 45 ppm group. This finding suggests that the Test Material does not cross the placenta in amounts tha are toxic to the fetuses. Table II shows mean birth weights of individual pups for all litters; these data likewise suggest that Test Material does not cross the placenta in amounts that produce weight loss in the pups.

The reductions in the indices concerning the pups themselves, e.g., Viability and Lactation, strongly suggest that maternal milk supply is either curtailed or that Test Material enters the milk and affects the pups, particularly in the 45 ppm group and to a lesser extent in the 15 ppm group. We believe that the slight reduction in the 5 ppm F2B Lactation and Viability indices, although statistically signicant, are not indicative of toxicity because the reductions are small (less than 10 % of Control values) and do not occur in 45 ppm F2B groups.

Table III shows mean total litter weight at weaning for each group. We calculated Student's "t" tests comparing treated groups with their corresponding control groups. The data in this Table show that significant weight loss occured only in the F1A and F2A litters at 15 ppm and in all litters at 45 ppm, except the F1B litter, which had only three litters remaining at weaning. This group had only 18 pups when weaned.

The findings of systemic toxicity in the 45 ppm adults, and the clearly demonstrated effects on offspring survival at 45 and 15 ppm indicate that the NOEL for this study is 5 ppm.

TABLE I

INDICES OF REPRODUCTION

DOSE, PPM

Index	Generation	0.0	5.0	15.0	45.0
Fertility	F1A	91.7	95.7	90.0	86.4
	F1B	91.7	95.8	85.7	83.3
	F2A	91.7	100.0	87.5	100.0
	F2B	87.5	91.7	95.7	80.0
Gestation	F1A	100.0	100.0	100.0	100.0
	F1B	100.0	100.0	100.0	93.3
	F2A	100.0	100.0	100.0	100.0
	F2B	95.2	100.0	95.5	75.0
Live Birth	F1A	99.6	100.0	96.1	95.4
	F1B	99.6	95.3	99.4	100.0
	F2A	98.8	100.0	100.0	100.0
	F2B	99.6	99.2	98.6	100.0
Viability	F1A	96.8	93.9	86.6*	38.7**
	F1B	98.3	97.3	98.9	31.6**
	F2A	98.1	95.9	97.8	81.4**
	F2B	95.5	90.1*	88.6*	100.0
Lactation	F1A	96.6	93.4	96.4	27.4**
	F1B	97.6	98.8	91.4*	46.2**
	F2A	98.3	94.1	88.7**	72.4**
	F2B	93.0	83.6*	89.8	90.9

* Significant at a = 0.05
** Significant at a = 0.01

$$\text{Fertility Index} = \frac{\#\text{ Gravid dams}}{\#\text{ Dams mated}} \times 100$$

$$\text{Gestation Index} = \frac{\#\text{ Dams with 1 or more live pup}}{\#\text{ Gravid dams}} \times 100$$

$$\text{Live Birth Index} = \frac{\#\text{ Viable pups at birth}}{\#\text{Pups born}} \times 100$$

$$\text{Viability Index} = \frac{\#\text{ Pups alive at day 5}}{\#\text{ Pups born}} \times 100$$

$$\text{Lactation Index} = \frac{\#\text{ Pups at weaning}}{\#\text{ Pups at cull}} \times 100$$

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TABLE II

MEAN PUP BODY WEIGHTS AT BIRTH (GMS)

Litter	0.0 PPM	5 PPM	15 PPM	45 PPM
F1A	5.8	5.7	5.9	5.4
F1B	5.7	5.8	5.9	5.2*
F2A	5.7	5.7	5.7	5.4
F2B	5.8	5.9	5.6	5.5

* Significant at $p \geq 0.05$. Note: This value is not considered to be toxicologically significant since it is confined to only one group.

TABLE III

MEAN TOTAL LITTER WEIGHT AT 4 WEEKS, GM

DOSE, PPM

Litters	0 PPM	5 PPM	15 PPM	45 PPM
F1A	453.2	433.0	373.0**	193.5**
F1B	399.9	421.0	355.0	267.3(a)
F2A	464.1	420.3	381.0**	203.0**
F2B	431.9	375.4	391.9	307.7* (a)

* Significant at $p \geq 0.05$ (Student's "t" test).
** " $p \geq 0.01$ " "

(a) Based on only three litters remaining.