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Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

007376

Study Type: Teratology

Tox. Chem. No.: 535

Accession No.: 260123

MRID No.: 152569

Test Material: Malathion

Synonym: AC6,601

Study Number: 8171

Sponsor: American Cyanamid Company
Princeton, NJ

Testing Facility: Food and Drug Research Labs., Inc.
Waverly, NY

Title of Report: A Teratology study with AC6,601 in rabbits.

Author(s): Joseph C. Siglin, Kenneth A. Voss, Peter J. Becci

Report Issued: February 28, 1985

*Study completed ~~10/15/84~~
10/15/84*

Special Review Criteria

A. Materials

1. Test Compound: Malathion, Description: clear brown to colorless liquid, Lot #: AC 4661-38, Purity: 92.4%

Contaminants: Not indicated.

2. Test Animals: Species: Rabbit, Strain: New Zealand White, Age: young, mature, Weight: 3.3 kg
Source: New York State Rabbit Development, Hartwick, NY



B. Study Design

The study design is based in part upon the results obtained in a range-finding study (Study No. 8170, February 28, 1985, MRID 152569). In the range-finding study, five rabbits (NZW-inseminated females) per group were exposed by the oral route to malathion in corn oil at doses of 25, 50, 100, 200, and 400 mg/kg body weight during days 6 to 18 of gestation. Mortality rates of 40 percent and 80 percent were observed in the 200 and 400 mg/kg dose groups, respectively. Additional toxic responses in the two high dose groups including salivation and/or tremors at the time of death. There were no deaths or other outward signs of toxicity noted at doses of 0 to 100 mg/kg. There were no fetal abnormalities observed at any dose.

As a result of the range-finding study, the dosages of malathion selected for the complete ceratology study were 0, 25, 50, and 100 mg/kg body weight.

1. Animal Assignment - Animals (pregnant does) were assigned 20/group to the following test groups:

Test Solutions

<u>Test Group</u>	<u>Treatment Level (mg/kg bwt)</u>	<u>Malathion Concentration (mg/mL)</u>	<u>Dosage Volume (mL/kg bwt)</u>
A	corn oil	0	1.5
B	25	16.7	1.5
C	50	33.3	1.5
D	100	66.7	1.5

Each mated doe received a single daily oral dose of the vehicle (corn oil) or malathion dissolved in corn oil on days 6 to 18 of gestation.

2. Compound Preparation: A stock solution of the test material was prepared by dissolving malathion in corn oil at a concentration of 66.7 mg/mL (high dose). The remaining test solutions (mid and low dose) were prepared by serial dilution of the stock solution with corn oil. Test solutions were stored at 0 to 5 °C and warmed to room temperature prior to use.

Experimental Procedures

All animals were individually housed in wire mesh cages and were fed and watered ad libitum. Animal Feed A, certified diet (Zeigler Bros., Gardner, PA), was utilized. (The following is paraphrased or quoted from pages 8 to 11 of the actual study.)

Following acclimation, male-female matings (1:1 pairings) were allowed. Observed copulation was designated as day 0 of gestation. Males were restricted to a single successful mating per day. Each mated female was given 200 IU of human chorionic gonadotropin by intravenous injection (ear vein). Bred does were randomly assigned to one of the four experimental groups.

Each doe was administered via gastric intubation a single daily oral dose of the vehicle (corn oil) or malathion dissolved in corn oil on days 6 to 18 of gestation. (Note: According to the Guidelines, day 0 corresponds to the day of mating; compound is supposed to be administered on days 7 to 19 of gestation.) All doses were administered in a volume of 1.5 mL/kg body weight, based on body weight on gestation day 6.

"Does were observed twice daily (at least 5 hours apart) for mortality. Detailed observations for outward signs of toxicity, either physical or behavioral, were conducted once daily.

"Individual body weight was measured on days 0, 6, 12, 15, 18, 24, and 29 of gestation. Body weight gain was individually calculated for days 0 to 6, 6 to 12, 6 to 18, 18 to 29, and 0 to 29. Corrected body weight (body weight minus gravid uterus weight) and corrected body weight gain were individually calculated for pregnant does sacrificed at study termination (gestation day 29).

"Does showing signs of abortion were immediately sacrificed by intracardiac injection of sodium pentobarbital and subjected to complete gross necropsy. The urogenital system of each animal was examined in detail for possible abnormalities and reproduction data (listed below) were obtained where possible. Does dying spontaneously were examined in this same manner. Surviving does were euthanized (i.c. sodium pentobarbital) on day 29 of gestation and subjected to complete gross necropsy. The urogenital system of each female was examined in detail for possible abnormalities. The ovaries and uterus were excised, the uterus was weighed and its contents then examined. The following data were recorded for each pregnant female:

- Number of corpora lutea.
- Number and position of implantation and resorption sites.

- Number and position of live and dead fetuses.
- Sex (by internal inspection of gonads), body weight, and crown to rump length of each live fetus.

"Uteri which appeared nongravid were opened and placed in 10% aqueous ammonium sulfide solution to detect possible early resorption sites and thereby confirms pregnancy status. All grossly abnormal maternal tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination. Fetuses were examined for the presence of external abnormalities and then carefully dissected and examined for the presence of visceral abnormalities. Soft tissue examination included gross evaluation of all major tissues and organs (cross sectioned). The brain of each fetus was examined by mid-coronal slice. Following visceral examination, each fetus was completely eviscerated, skinned, tagged (ID number) and placed in 70% isopropyl alcohol. After complete fixation, fetuses were processed for skeletal examination. Specifically, this procedure involved macerating in 2% aqueous potassium hydroxide solution, staining with Alizarine Red S (Dawson, 1926), and clearing in 20%, followed by 50% aqueous glycerine solution."

Statistical Analysis

"Maternal body weight data were analyzed using analysis of variance. Differences between the control and test groups were determined using the Least Significant Differences Test (Snedecor and Cochran, 1967). Incidence data such as group fertility ratios, daily observation data, maternal gross necropsy data, and the number of does with two or more resorption sites (per group) were evaluated using the Fisher's Exact Probability Test (Siegel, 1956). For appropriate reproduction data, the litter was used as the experimental unit of measurement. Reproduction data (corpora lutea count, number of implantation sites, number of resorption sites, percent resorptions, number of fetuses, number of live fetuses, number of dead fetuses, number of stunted fetuses, litter sex ratios, average live litter weight, and crown to rump length data) along with fetal external, visceral, and skeletal examination data were evaluated using the Kruskal-Wallis Test. Differences between the control and test groups were determined using the Mann-Whitney U Test (Siegel, 1956). Significance was judged at a level of $p \leq 0.05$ for all statistical evaluations.

"The above methods for data evaluation differ slightly from those specified in the study protocol. These methods are routinely used by this laboratory for evaluation of such data. Approval to use these procedures was given by the sponsor's representative, Dr. N. Luke on December 21, 1984" (pages 8-11).

Quality Assurance:

Quality assurance was affirmed by Frederick F. Paul, Quality Assurance Unit, in accordance with GLP Regulations, Appendix XI, page 134.

Results

Daily Observations: Daily observations of does included responses commonly associated with cholinesterase inhibition, such as anorexia, nasal discharge, diarrhea, soft stools and decreased activity. Examination of these data demonstrate that control does responded in a similar manner as dosed females with the possible exception of anorexia and soft stools in the high dose group. Nasal discharge is difficult to interpret as presented since all groups had females responding prior to, during and after dosing, in other words, throughout the study. While there are an average of 1-2 more females per dose group with nasal discharge than control females, there is no clear evidence that this was compound related. (p. 18-21)

Mortality: No dose-related increase in mortality was observed. Two does which died in the high dose group were attributed to accidental intubation into the lung during dosing. There were four premature deaths in the low-dose and three deaths in the mid-dose, but these are not considered to have been related to any adverse effects of malathion. The causes of death in the low- and mid-dose group were not revealed at necropsy. It is noted that, with one exception, all other animals died during the dosing period.

Abortion: There was no evidence of an effect of the compound in promoting abortion.

Body Weight: Body weight data during 29 days of gestation, as summarized in Table 2 (p. 22) does not reveal any remarkable effects of dosing with malathion. No statistically significant changes were reported.

The corrected body weight gain data for days 0 to 29 (Table 3 p. 23), reveal a trend toward reduced body weight gain with increasing dose. It should be mentioned at this point that Appendix III which reportedly contains the individual data is missing from the document and, hence, TB is unable to verify or validate the findings.

Corrected Body Weight Gain (kg) Days 0-29	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
	0.23±0.22	0.20±0.19	0.14±0.16	0.11±0.21

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During days 6 to 18 of the gestation period body weight gain at the mid and high doses are reported as significantly less than the control, P < 0.05.

	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
Body Weight Gain (kg), Days 6-18	0.19±0.21	0.06±0.33	-0.03±0.30	-0.03±0.27

The numerical reduction in body weight gain between control and low dose groups is not statistically significant during this period of dosing (days 6-18). This deficit is principally accounted for during days 6-12. For days 12-18, low dose dams actually gain more weight than control, thus demonstrating the transient nature of the response. However, the standard deviation for all groups is equivalent in magnitude to the body weight gain thereby reducing the reliability of these measurements. The additional data which may be contained in Appendix III is therefore necessary to evaluate the effect, if any, on body weight gain.

Live fetal weight did not appear to be affected by dosing (Table 4, p. 25).

	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
Live Fetal Weight (g):	41.3	41.4	37.3	40.4

Reproduction and Gestation: The only adverse effect observed was the number of resorptions per doe. There were increases in the mean number of resorption sites and mean percent of resorption sites per doe tabulated as follows (from Table 4, page 24):

	<u>Treatment Level (mg/kg bwt)</u>			
	<u>Control</u>	<u>25</u>	<u>50</u>	<u>100</u>
Resorption Sites	0.9±1.2	0.7±1.1	2.3±2.8	2.0±2.7
Percent Resorptions	15.6±26.9	12.3±20.7	29.2±34.2	28.4±34.9

In accordance with the Agency's Guidelines for the Health Assessment of Suspect Developmental Toxicants (FR 51: September 24, 1986, p. 34031), the increases in these parameters at the mid and high doses are to be viewed as evidence of developmental toxicity. Hence, developmental NOEL = 25 mg/kg/day.

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Gross Necropsy of Does: No adverse effects observed.

Fetal External Examinations: There was no evidence of any compound-related fetal external effects.

Fetal Soft Tissue Examination: There was no evidence of any compound-related fetal soft tissue effects.

Fetal Skeletal Examination: There was no evidence of any malathion-related fetal skeletal effects with respect to either malformations or developmental variations as defined in the study (page 16).

Conclusions

TB concurs with the conclusions enunciated in the study, namely, that daily oral dosing with malathion at 25, 50, or 100 mg/kg body weight daily during the period of organogenesis (days 6-18) provided no evidence of any teratogenic effect in New Zealand White rabbits. Maternal toxicity, evidenced by reductions in doe body weight gains, occurred with statistical significance at the mid and high dose. Hence, for this parameter NOEL = 25 mg/kg/day. The only additional sign of toxicity was a slight increase in mean resorptions per doe at the mid and high doses, considered as evidence of developmental toxicity.

Core NOEL = minimum, conditional upon receipt of Appendix III

Developmental NOEL = 25 mg/kg/day

Maternal NOEL = 25 mg/kg/day

Maternal LEL = 50 mg/kg/day (reduced doe body weight gain during period of gestation)

A/D = 1