3/1/1994

FINAL

DATA EVALUATION REPORT

MK-0243

Study Type: Subchronic Oral Toxicity in Mice

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity in Mice (Guideline Series 82-1a)

EPA IDENTIFICATION NUMBERS

Tox Chem. Number: New chemical

MRID Number: 427436-21

PC Number: 122806

TEST MATERIAL: MK-0243

SYNONYM(S): L-656,748, deoxy avermectin

SPONSOR: Agricultural Research and Development, Merck Research Laboratories, Three Bridges, NJ

STUDY NUMBER: Laboratory Project Identification 618-244-TOX18 (TT#90-061-0)

TESTING FACILITY: Merck Research Laboratories, West Point, Pennsylvania

TITLE OF REPORT: MK-0243: Thirteen-Week Dietary Toxicity Study in Mice. TT #90-061-0

AUTHOR: Ronald Gerson

STUDY COMPLETED: December 18, 1992

QUALITY ASSURANCE: A signed Certification of Good Laboratory Practice, a signed Quality Assurance Statement, and a list of Quality Assurance inspections were included in the report.

CONCLUSIONS: Male and female Crl CD-1 mice were fed diets containing MK-0243 for 13 weeks. Dietary levels were adjusted weekly to provide constant intake levels of 0, 0.5, 4.5, or 15.0 mg/kg/day of MK-0243. Also, one additional group of mice received a dose level of 1.5 mg/kg/day for the first 7 weeks and 10 mg/kg/day for the remaining 6 weeks to give a time-weighted average dose of 5.4 mg/kg/day.

NOEL = 5.4 mg/kg/day (TWA)

LOEL = 15.0 mg/kg/day based on decreased mean body weight and decreased cumulative mean body weight gain (marginal in females)

CORE CLASSIFICATION: Core Supplementary. This study does not satisfy the guideline requirements for a rodent subchronic oral toxicity study (82-1a) for the following reasons: several recommended clinical chemistry parameters were not examined and the study failed to perform complete histopathological analyses on all control and high-dose animals. However, a new study is not required because an acceptable rodent (rat) subchronic study was performed and because this study was intended primarily as a range-finding study for the mouse oncogenicity study.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Test material: MK-0243, a 4'-epi-methylamino-avermectin derivative with pesticidal activity
Batch number: L-656,748-038W002
Stability: Analysis by HPLC of the amount of test material in the diet at 1, 8, and 12 weeks indicated that the test material was stable for the duration of this study.
Storage: Room temperature
Purity of bulk test material: HPLC analysis before study initiation and at termination "confirmed the purity of the test article was 91.1 percent B_{1a} and 5.1 percent B_{1b}."
Vehicle control: Certified Purina Rodent Chow Meal

Note: A factor of 1.14 was used in test material calculations since the test material MK-243 was supplied as a benzoate salt.

2. Animals

Species: Mice
Strain: Crl:CD-1(ICR)BR
Source: Charles River Laboratories, Raleigh, NC
Age (at study initiation): Approximately 6 weeks
Body weight (at study initiation): 23.3-30.7 g. for males; 19.1-26.3 g. for females
Animals/dose: 15/sex/dose
Route: Oral via diet
Total number of doses: Animals received the test material or vehicle in the diet for at least 84 days
Dose levels:
Group 0 - vehicle control
Group 1 - 0.5 mg/kg/day test material
Group 2 - 1.5 mg/kg/day (weeks 1 - 7) and 10 mg/kg/day (weeks 8 - 13) test material = 5.4 mg/kg/day (TWA)
Group 3 - 4.5 mg/kg/day test material
Group 4 - 15.0 mg/kg/day test material

Temperature: Not reported
Relative humidity: Not reported

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Photoperiod: 12 hours
Housing: 3 mice per box
Feeding: Certified Purina Rodent Chow Meal *ad libitum* (feed was withdrawn on the day prior to scheduled bleeding/necropsy)
Water: *Ad libitum*
Identification: Tattoo and ear notches
Acclimation: Not reported
Selection: Random, only healthy animals without ophthalmologic abnormalities were used

3. Test procedure

Mice in Group 0 received Certified Purina Rodent Chow Meal *ad libitum*, and mice in Groups 1, 2, 3, and 4 each received 0.5-, 1.5- (increased to 10 mg/kg/day at week 8), 4.5-, or 15.0-mg/kg/day MK-0243 mixed with Certified Purina Rodent Chow Meal *ad libitum* for at least 84 days.

4. Diet Preparation and Analyses for Purity and Stability

Test diets containing MK-0243 were prepared weekly by mixing the test material with the basal diet (Certified Purina Rodent Chow Meal). The concentration of test material in the feed at each dietary level was adjusted weekly based on body weight and feed consumption data from the most recent week to maintain a fairly constant intake of test material/kg body weight. In each dose group, separate batches of test diet were prepared for the males and females.

Homogeneity and actual concentrations of the test material in the diet were determined using HPLC analysis. Homogeneity of the diet was calculated at weeks 1 and 8; three samples were taken from the diets (samples were: top, middle, and bottom). The concentration and percent nominal values were determined for weeks 1, 8, and 12. Uniformity was considered to be acceptable by the study author. The coefficient of variation was less than for all batches examined. The average percent nominal value for all the submitted samples was 85.4±3.5% (n=56); the range was 80.3-93.9%. Although none of the values fell below 80%, by guideline standards, the achieved concentrations are low. Average concentrations should be within 10% of target.

For week 1 samples, the vehicle control had values of 0.11 ppm for males and <3.75 ppm for females. At weeks 8 and 12, the values for both males and females were <0.10 ppm (the limit of detection was 0.10 ppm). The study author did not consider the low test material concentration in the vehicle at 1 week to be significant since it was approximately 20 times lower than the low-dose group.

5. Statistical Methods

Statistical analyses were not presented.

6. General Observations

(a) Mortality/moribundity/survival

Animals were observed daily for mortality and moribundity. One high-dose female died during week 5, and one high-dose male died during week 10. The cause of death was not determined for either animal, and no clinical signs were reported. The possibility that the deaths were treatment related could not be ruled out.

(b) Clinical signs

Animals were observed daily for clinical signs; less extensive examinations were made on the weekends and on holidays. Average individual food consumption was calculated weekly.

Results: Individual animal data for clinical signs were not reported. The study authors indicated that no treatment-related clinical signs were observed, but since individual animal data were not provided, this could not be verified by the reviewers.

(c) Body weights/food consumption/test material intake

Body weights--Animals were weighed pretest and once weekly thereafter.

Results: For the 15-mg/kg/day group, the mean body weight at 12 weeks was 95% of control for females and 89% of control for males. For males (but not females) at 15 mg/kg/day, there was a statistically significant decrease in mean body weight at 8 and 12 weeks.

For the 15-mg/kg/day group, the cumulative mean body weight gain at 12 weeks was 79% of control for females and 59% of control for males. For males at 15 mg/kg/day, cumulative mean body weight gain was significantly decreased. There were no significant changes in mean body weight or cumulative mean body weight gain for the 0-, 0.5-, 1.5/10-, or 4.5-mg/kg/day groups. Refer to Table 2 for body weight data.

Note: Since no decrease in body weight gain was apparent in the 1.5-mg/kg/day group by week 7, the dose was increased to 10 mg/kg/day for the remainder of the study.

Food consumption--Food consumption for each cage was measured once weekly over a 6-day interval (except for females during week 1 when a 5-day interval was used). Individual food consumption values for each mouse were obtained by dividing the food consumption/cage by the number of mice/cage.

Results: No significant changes in food consumption were reported. A decrease in mean food consumption during week 2 was noted for males in all treatment groups and for females in Groups

1, 2, and 4; this was attributed to decreased access to rodent chow caused by a technical error.

Total mean food consumption values for females were 5.2, 5.0, 4.7, 5.0, and 4.9 g/day for groups 0, 1, 2, 3, and 4, respectively. Total mean food consumption values for males were 5.0, 4.9, 4.9, 4.8, and 4.6 g/day for groups 0, 1, 2, 3, and 4, respectively.

Note: Some individual food consumption values were not reported and not included in the calculations of mean food consumption or test material intake because of observed or suspected food spillage.

Test article intake--Mean individual test article intake (mg/kg/day) was calculated by the study author (method unspecified; however, the dietary concentrations of test material, individual food consumption data [based on cage consumption values], and body weight data were reported) (Table 2).

Table 2. Mean Test Article Intake

Nominal Dose (mg/kg/day)	Mean Test Article Intake[a] (mg/kg/day) Females	Males
0.5 (weeks 1-12)	0.52 (0.40-0.70)	0.49 (0.38-0.74)
1.5 (weeks 1-7)	1.53 (1.12-1.98)	1.46 (1.24-1.84)
4.5 (weeks 1-12)	4.70 (3.80-6.08)	4.46 (3.33-6.20)
10.0 (weeks 8-12)	10.38 (8.76-11.02)	10.50 (8.92-13.96)
15.0 (weeks 1-12)	15.58 (12.28-20.98)	15.15 (11.96-21.00)

[a]Numbers in parentheses indicate the range of values. Data taken from Study TT #90-061-0, Table A-5.

(d) Ophthalmoscopic examination

Ophthalmic examinations were conducted on all animals at pretest and on control and high-dose animals during week 12.

Results: No treatment-related changes were observed in any of the control or high-dose animals at the terminal examination.

7. Clinical Pathology

At study termination, hematological and blood chemistry analyses were performed for all surviving animals. Animals were fasted overnight prior to blood collection. A caval blood sample was obtained following anesthetization with ether vapor. The parameters marked (X) below were examined.

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)

* Recommended by Subdivision F (November 1984) Guidelines

Results: No significant treatment-related changes were reported.

(b) Blood (clinical) chemistry

Electrolytes

Calcium*
Chloride*
Phosphorus*
Potassium*
X Sodium*

Enzymes

Alkaline phosphatase (ALP)
Creatinine phosphokinase
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other

X Albumin*
Blood creatinine*
X Blood urea nitrogen*
Cholesterol
X Albumin/Globulin ratio
X Glucose*
Total bilirubin*
Triglycerides
X Total protein*

* Recommended by Subdivision F (November 1984) Guidelines

Results: No treatment-related findings were reported for albumin-to-globulin ratio, alanine aminotransferase, aspartate aminotransferase, serum protein, albumin, glucose, urea nitrogen, or sodium.

Note: Assays for bilirubin, serum creatinine, potassium, chloride, cholesterol, calcium, phosphorus, triglycerides, and alkaline phosphatase were planned in the study protocol but were not performed for any animal because of insufficient serum sample size.

(c) Urinalysis

Urinalysis was not performed.

8. Sacrifice and Pathology

Prior to necropsy, animals were fasted overnight. Complete gross examinations were performed on all animals (15/sex/group). All tissues were preserved in 10% buffered neutral formalin or Bouin's solution. Tissues (marked with an X below) from five high-dose

animals/sex and five control animals/sex, from all mice dying before scheduled necropsy, and from all gross lesions were examined histologically using paraffin-embedded tissue sections stained with hematoxylin and eosin. The organs indicated by XX below were also weighed for all animals.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Peripheral nerve (sciatic)*
Esophagus*	X Bone marrow*	X Spinal cord
X Stomach*	X Lymph nodes*	
X Duodenum*	XX Spleen*	X Pituitary*
X Jejunum*	X Thymus*	X Eyes (incl. optic nerve#)*
X Ileum*		
X Cecum*	Urogenital	Glandular
X Colon*		
X Rectum*	XX Kidneys*	X Adrenals*
XX Liver*	X Urinary bladder*	Lachrymal gland
X Gallbladder*	XX Testes*	X Mammary gland##
X Pancreas*	X Epididymides	X Thyroids*
	X Prostate	X Parathyroids*,###
Respiratory	Seminal vesicle	X Harderian glands
	X Ovaries	
Trachea	X Uterus*	
X Lung*		

Other

X Bone
X Skeletal muscle
X Skin
X All gross lesions* and all ophthalmologic lesions

* Recommended by Subdivision F (November 1984) Guidelines
\# When present in the eye section
\## When present in the skin section
\### When present in the thyroid section

(a) Macroscopic

No treatment-related effects were reported.

(b) Organ weights

No treatment-related changes were reported in absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio.

(c) Microscopic

No treatment-related effects were reported.

B. DISCUSSION

At the highest dose tested, 15 mg/kg/day, both males and females were reported to exhibit a decrease in body weight gain that was not related to decreases in food consumption. The weight gain in high-dose males was 59% of control and was statistically significant (determined by the reviewers). The weight gain in high-dose females was 79% of control, but was not statistically significant (determined by the reviewers). Although the decrease in body weight gain in females was not statistically significant, it was considered by the reviewers to represent a marginal effect. However, for purposes of determining a maximally tolerated dose, the high dose in females may not be adequate.

In addition, two deaths (cause of death unknown) were reported in the high-dose group; it is unknown whether they were compound related.

Based on the evidence of decreased body weight, the LOEL for this study was determined to be 15 mg/kg/day. The NOEL was 5.4 mg/kg/day (TWA).

Several deficiencies were noted in the design of the study. For example, clinical chemistry was not performed on several electrolytes, blood creatinine, or total bilirubin as recommended in the guideline. The guideline also recommends that full histopathology of all animals in the control and high-dose groups should be done; however, histopathology was performed on only 5 high-dose animals/sex and 5 control animals/sex, and all animals that died during the study. Based on these deficiencies, the study is classified as Supplementary. An additional study in mice is not required because this study was intended only as a range-finding study for an oncogenicity study and an adequate rodent (rat) study was performed.

In addition, a number of other deficiencies were noted in the study report that would not be expected to affect the study conclusions. These included failure to report the acclimatization period, the temperature or humidity of the animal room, or the description of the test material (i.e., color, state). Also, the ophthalmology results were reported to be negative, but this report was not included.

Table 2. Mean Body Weight (grams) and Cumulative Mean Body Weight Gain of Mice Exposed to MK-0243 for 13 Weeks[a,b]

Treatment Group	Mean Body Weight				Cumulative Mean Bodyweight Gain
	-1 weeks	4 weeks	8 weeks	12 weeks	
Control					
Female	22.7	24.7	27.6	29.8	7.1
Male	26.5	31.4	33.6	37.3	10.8
0.5 mg/kg/day					
Female	22.9(101%)	25.2(102%)	26.8(97%)	29.6(100%)	6.7(94%)
Male	26.6(100%)	32.5(103%)	32.8(98%)	38.2(102%)	11.7(108%)
1.5/10 mg/kg/day[c]					
Female	23.1(102%)	25.7(104%)	27.5(100%)	29.7(100%)	6.7(94%)
Male	26.8(101%)	33.4(106%)	33.6(100%)	39.0(105%)	12.2(113%)
4.5 mg/kg/day					
Female	23.2(102%)	24.3(98%)	27.5(100%)	30.2(101%)	7.1(100%)
Male	27.2(103%)	32.4(103%)	33.3(99%)	37.7(101%)	10.5(97%)
15.0 mg/kg/day					
Female	22.7(100%)	22.8(92%)	25.2(91%)	28.4(95%)	5.6(79%)
Male	26.9(102%)	29.2(93%)	28.3(84%)*	33.3(89%)*	6.4(59%)*

[a]Data abstracted from the study report, Tables A-1, A-2, A-13, and A-14.
[b]Numbers in parentheses indicate percent of control value.
[c]1.5 mg/kg/day for weeks 1-7, 10 mg/kg/day for weeks 8-13
*Significant at $p \leq 0.01$ by Scheffe's test and ANOVA, analysis by reviewers