FINAL

DATA EVALUATION REPORT

Deoxy Avermectin

Study Type: Subchronic Oral Toxicity in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

January 1994

Primary Reviewer Kate Rantz Date 1/17/94
Kate Rantz, M.P.H.

Independent Reviewer William L McLellan Date 1/17/94
William McLellan, Ph.D.

QA Reviewer John Liccione Date 1/17/94
John Liccione, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-133
Clement Number: 2-133/429 and 430
Project Officer: Caroline C. Gordon

EPA Reviewer: Linnea Hansen
Review Section IV, Tox Branch I
Health Effects Division

Signature: Linnea J. Hansen

Date: 2/3/94

EPA Section Head: Marion Copley
Review Section IV, Tox Branch I
Health Effects Division

Signature: Marion Copley

Date: 2/22/94

DATA EVALUATION REPORT

STUDY TYPE: Guideline series 82-1, subchronic oral toxicity in rats

CAS NUMBER:

TOX CHEM. NUMBER: New chemical

P.C. NUMBER: 122806

MRID NUMBERS: 427942-01 (main study), 427436-20 (range-finding study)

TEST MATERIAL: Deoxy avermectin

SYNONYMS: L-656,748
MK-0243

SPONSOR: Agricultural Research and Development
Merck Research Laboratories
Merck & Co., Inc.
Hillsborough Road, Three Ridges, New Jersey

STUDY NUMBER: TT #88-059-0 (main study)
TT #88-046-0 (range-finding study)

TESTING FACILITY: Merck Research Laboratories
Merck & Co., Inc.
West Point, Pennsylvania and Rahway, New Jersey

TITLE OF REPORT: L-656,748. Fourteen-week Dietary Toxicity Study in Rats

AUTHOR: George R. Lankas, Ph.D.

REPORT ISSUED: December 18, 1992

QUALITY ASSURANCE: A GLP certification statement, signed by the Study Director on December 18, 1992, was present. The study was conducted in compliance with GLP standards except that analysis of the stability of the active ingredient and characterization of the analytical standards were

performed following FDA GMP regulations. The test material was characterized in a non-GLP laboratory after it was used in the study.

CONCLUSION: Deoxy avermectin was administered orally via the diet to male and female CD rats (20/sex/dose) for 13 weeks at dietary levels of 0, 5, 25, or 125 ppm (0, 0.5, 2.5, or 12.5 mg/kg/day). Because of reduced body weight and food consumption observed at the highest dose level, the high dose was decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9. The time-weighted average high-dose levels were 6.7, 7.1, and 7.5 mg/kg/day for males, females, and sexes combined, respectively.

NOEL = 2.5 mg/kg/day

LOEL = 5 mg/kg/day. The LOEL is based on moribundity in high-dose males; neurotoxic effects including tremors, hindlimb splaying, and urogenital staining in both sexes; histological changes in the brain, spinal cord, sciatic and optic nerves, and skeletal muscle in males; and emaciation, reduced body weight, and reduced food consumption in high-dose males and females.

CORE CLASSIFICATION: Core Minimum. This study satisfies the guideline requirements (82-1) for a subchronic oral toxicity study in rodents. However, weekly laboratory analyses should be provided to verify stability, homogeneity, and actual concentration of the test material. Environmental conditions during the study, the acclimation period, and the method for assigning animals to study groups should be described in greater detail. Statistical analyses on individual animal data should be provided in the study report.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: MK-0243

Chemical formula: $C_{48}H_{72}O_{14}$

Batch number: L-656,748-010V003

Purity: 92.8% B_{1a} and 4.1% B_{1b} by HPLC, plus 0.76% by weight propyl gallate as antioxidant

Physical properties: Not provided

Stability: Stable in rodent feed for one week if stored at room temperature; expiration date not reported

2. Diet Preparation and Analysis

Test diets providing target dosage levels of 0.5, 2.5, and 12.5/8/5 mg/kg bodyweight/day were prepared weekly. Initial dietary levels were 5, 25, and 125 ppm test material. Dietary levels in ppm were adjusted at intervals during the study based on

body weight gain and food consumption data. Separate test diets were prepared for males and females in each dose group.

When reduced body weight and food consumption were observed early in the study, the high-dose level of 12.5 mg/kg/day was decreased to 8 mg/kg/day week at 3, and from 8 to 5 mg/kg/day at week 9. The 13-week time-weighted average dose for the high-dose groups (sexes combined) was calculated by the reviewer to be 7.5 mg/kg/day.

Purity was confirmed by HPLC before initiation of dosing and after completion of the study.

The analytical method used to determine stability, homogeneity, and actual concentration of the test material in the test diets was not described. Homogeneity tested at study initiation was acceptable, and ranged from 132–140 ppm (106–112% of target level) for the high-dose (125 ppm) group; 26–27 ppm (104–108% of target level) for the mid-dose (25 ppm) group; and 6 ppm (120% of target level) for the low-dose (5 ppm) group. Stability analyses conducted at weeks 1, 2, 3 (high-dose only), 8, and 14 were acceptable.

3. Animals

Healthy male and female Crl:CD®(SD) BR rats (20/sex/dose) from Charles River Breeding Laboratories, Inc., Wilmington, MA were randomly assigned to study groups. Rats were uniquely identified by tattoos and housed individually in steel cages in an environmentally controlled room with a 12 hour dark/light cycle. Animals were provided food (Purina Certified Rodent Chow Meal®) and water *ad libitum* throughout the study. At initiation of dosing rats were approximately five weeks old and weighed from 97 to 184 g (sexes combined).

The study authors did not provide other information about environmental conditions (e.g., temperature, humidity), criteria for assignment to study groups, or a description of the acclimation period.

Rationale for dose selection: Dietary levels of 5, 25, and 125 ppm for the current study were selected based upon the results of a 3-week dietary range-finding study in rats. The range-finding study was available for review (MRID no. 427436-20; study no. TT #88-046-0). In the range-finding study, rats received 5, 25, 50, or 100 ppm (0.5, 2.5, 5, or 10 mg/kg/day) in the diet for two weeks. The high dose level was increased from 10 to 20 mg/kg/day on day 8 of dosing. The reported LOEL was 10-20 mg/kg/day based on statistically significant decreases in leukocyte, lymphocyte, and neutrophil counts in females. Neurotoxicity, decreased food consumption, and decreased body weight gain were reported at dose levels of 10 to 20 mg/kg/day.
Additional details of the study can be found in Appendix B.

4. Statistical Analyses

Statistical analyses were not provided in the study report. Statistical methods used for data analyses were not described. The reviewers performed statistical analyses on selected parameters (i.e., body weight, food consumption, hematology, clinical chemistry, urinalysis, and absolute/relative organ weights) using Scheffe's test and ANOVA. Statistical analyses of the parameters that were not affected by treatment with the test material, or that appeared to be secondary to poor condition of high-dose animals, are contained in Appendix A, Tables A-1 through A-5.

5. General Observations

(a) Mortality/moribundity/survival

Rats were observed daily for mortality and moribundity.

Results - Nine high-dose (12.5/8/5 mg/kg/day) males were euthanized in weeks 3-11 because of treatment-related poor condition and emaciation. Dose levels for this group were 8 mg/kg/day in weeks 3 to 9, and 5 mg/kg/day in weeks 9 to 14. Necropsy revealed emaciation, decreased muscle mass, and microscopic changes in the brain, spinal cord, sciatic nerve, optic nerve, and skeletal muscle. No animals from the low- or mid-dose groups died or were moribund during the dosing period.

Non-treatment related deaths of one low-dose and two high-dose females (week 5) and one control female (week 8) resulted from anesthetic overdose during blood collection. In addition, one control and one mid-dose male were euthanized as a result of traumatic damage to the mouth from the metal food dispensers.

(b) Clinical observations

All rats were observed daily, and less rigorously on weekends, for general appearance, behavior, and overt signs of toxicity. The study author did not provide summary data for clinical observations in the study report.

Results - Table 1 summarizes the frequency of selected clinical observations in high-dose rats. Treatment-related clinical signs of toxicity included fine whole-body tremors, hind-limb splaying, and urogenital staining in high-dose males and females. In both sexes tremors began during week 1 (dose level 12.5 mg/kg/day). After the dose was decreased to 8 mg/kg/day in week 3, the frequency of tremors decreased briefly in week 4 before returning to previous levels. In week 9 the high dose was reduced to 5 mg/kg/day, after which the frequency of tremors decreased steadily. Hind limb splaying was observed in 4 high-dose males in week 7 and in

5

2 high-dose males in week 9. In weeks 11-13 the frequency of hind limb splaying was greatest, and was observed in 5 females and 8 males from the high-dose groups. Despite the reduction in high-dose levels, hind limb splaying occurred mostly near the end of the study period, which suggests cumulative or delayed neurotoxicity, or the onset of neurogenic atrophy of skeletal muscle. Urine staining was observed sporadically in males and less frequently in females in study weeks 2-14.

Tremors, hindlimb paresis, and urine staining (except for a single incidence in a mid-dose rat) were not observed in any control, low-, or mid-dose animals.

(c) Body weights/food consumption

Body weight/body weight gain--Individual body weights were measured before initiation of dosing, once weekly during the study period, and at termination.

Results - Selected body weight data are provided in Table 2. Changes related to treatment included mean body weights in high-dose groups that were significantly less than control values throughout the study ($p \leq 0.01$), beginning at week 1. As a result of excessively reduced body weight observed in both sexes, the high dose was decreased from 12.5 to 8 mg/kg/day during week 3, and from 8 to 5 mg/kg/day during week 9. Mean body weights of high dose animals were 61-83% of controls. No treatment-related changes in body weight in low- and mid-dose rats were observed.

Food consumption--Food consumption was measured weekly over a six day period.

Results - Food consumption data are reported in Table 3. Treatment-related, statistically significant decreased food consumption was sustained in high-dose males and females throughout the dosing period. Food consumption in high-dose groups (sexes combined) was 72-90% of controls.

Test article intake--Target dosage levels of 0, 0.5, 2.5, and 12.5/8/5 mg/kg/day corresponded to initial dietary concentrations of 0, 5, 25, and 125/80/50 ppm test material. Table 4 summarizes intake of the test material over 13 weeks.

Table 4. Average Intake of the Test Material over 13 Weeks

Target Dose Level (mg/kg/day)	Weekly Average Intake (mg/kg/day)[a] Males	Females	Range
Low (0.5)	0.5	0.5	0.4–0.6
Mid (2.5)	2.5	2.6	2.1–2.9
High (12.5/8/5)			
weeks 1–2 (12.5)	11.0	11.6	not reported
weeks 3–8 (8)	6.8	7.3	not reported
weeks 9–13 (5)	4.8	5.1	not reported
weeks 1–13	6.7	7.1	not reported

[a] Data extracted from page 17 of the study report.

(d) Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on control and high-dose rats in study weeks 4 (both sexes), 10 (males), and 11 (females). An additional exam was conducted during week 13 on any animal that appeared to have been traumatized when blood was drawn from the orbital sinuses.

Results - No treatment-related effects were observed. A complete cataract was found in one eye of a single high-dose female in the week 13 examination. The study authors stated that the relationship of the cataract to treatment with the test material was uncertain – cataracts were not observed in any other treated animal and the historical incidence of cataracts was about 0.5% (historical data not provided in the study report).

6. Clinical Pathology

Blood for hematology and clinical chemistry tests was collected from 15 males and 15 females from each dose group in test weeks 5, 8, and 12. Animals were fasted 16-19 hours (up to 24 hours at scheduled sacrifice). Blood was drawn from the orbital sinuses and tail veins of fasted animals under ether vapor anesthesia. The hematology and blood chemistry parameters indicated below by an "X" were examined:

(a) Hematology

X Clotting time
X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte total count (WBC)*
X Leukocyte differential count*
X Erythrocyte count (RBC)*
X Platelet count*
X Mean corpuscular hemoglobin
X Mean corpuscular volume
X Mean corpuscular hemoglobin concentration

*Recommended by Subdivision F (November 1984) Guidelines

Results - Selected hematology data are presented in Table A-1 of Appendix A. No treatment-related changes were seen. High-dose males had slight but statistically significant increased erythrocyte counts, hemoglobin levels, and hematocrit values compared to controls at some sampling times, although the values were within the normal range. High-dose males also had slightly decreased leukocyte, lymphocyte, and neutrophil counts, although these decreases were not significantly different from control values. No significant changes in hematology parameters were observed in low- and mid-dose males or in females at any dose level. The slightly increased erythrocyte counts, hemoglobin levels, and hematocrit values may have been secondary effects resulting from treatment-related decreased food consumption and dehydration in high-dose animals (although no water consumption data were provided, urinary volume was markedly decreased in high-dose animals).

(b) Blood (clinical) chemistry

Electrolytes

Calcium*
X Chloride*
Phosphorus*
X Potassium*
X Sodium*

Other

X Albumin*
X Albumin/globulin ratio
X Creatinine*
X Blood urea nitrogen*
X Cholesterol
X Glucose (fasting)*
X Total protein*
Total bilirubin*
X Triglycerides

Enzymes

X Alkaline phosphatase (ALP)
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*

*Recommended by Subdivision F (November 1984) Guidelines

Results - Selected clinical chemistry data are presented in Table A-2 of Appendix A. No treatment-related changes were

8

observed. Statistically significant ($p \leq 0.01$) decreased glucose and creatinine levels were observed in high-dose males and females in weeks 5, 8, and 12. Other trends observed in high-dose groups included decreased total protein and increased blood urea nitrogen and A/G ratios. The decreased creatinine levels and other parameters were likely to be secondary to decreased muscle mass and general emaciation observed in high-dose animals.

(c) Urinalysis

Urine was collected from fasted animals (10/sex/group) during test weeks 8 and 12. Parameters marked below by an "X" were examined.

X Protein	X Specific gravity
X Glucose	X Ketones
X Bilirubin	X Urobilinogen
X Occult blood	X Urine volume
X pH	X Urinary sediment

*Recommended by Subdivision F (November 1984) Guidelines

Results - Selected urinalysis parameters are shown in Table A-3 of Appendix A. Effects observed appeared to be secondary to the generally poor condition (emaciation, dehydration) of the treated animals. High-dose males had significantly decreased urine volume and significantly increased urine specific gravity. Similar trends were observed in high-dose females, although the values were not significantly different from controls.

7. Sacrifice and Pathology

Complete gross examinations were conducted on all rats at terminal sacrifice after exsanguination of the fasted animals under carbon dioxide anesthesia. Tissues from high-dose and control animals, and from rats with unscheduled deaths, marked with an "X" below were examined histologically. Tissue from bone, skeletal muscle, brain, spinal cord, sciatic nerve, eyes, and gross lesions were examined microscopically for all rats. Tissues marked by "XX" below were weighed for all animals.

Respiratory	Cardiovascular/ Hematologic	Nervous
Nasal tissues*	XX Heart*	XX Brain*
Trachea*	Aorta*	X Peripheral n.*
XX Lungs*	XX Thymus*	X Spinal cord
	X Bone Marrow*	Endocrine
Digestive	X Lymph nodes*	XX Adrenal glands*
X Salivary glands*	XX Spleen*	XX Thyroid glands*
Esophagus*		X Parathyroids*
X Stomach*	Urogenital	XX Pituitary*
X Duodenum*	XX Kidneys*	
X Jejunum*	X Urinary Bladder*	Other
X Ileum*	XX Testes/ovaries*	X Tissues with gross lesions*
Cecum*	XX Uterus/prostate*	X Bone*
X Colon*		X Eyes*
X Rectum*		X Lymph node*
XX Liver*		X Skin
X Pancreas*		X Mammary gland
Gall bladder*		

* Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic

Treatment-related gross changes are summarized in Table 5. Emaciation and skeletal muscle atrophy (primarily evident in the muscles of the hind leg) were observed in high-dose males and females.

(b) Organ weight and body weight ratios

Absolute and relative organ weight data (with statistical analysis was performed by the reviewer) are presented in Tables A-4 and A-5 of Appendix A. No treatment-related changes in absolute or relative organ weights were observed. Absolute organ weights in high-dose males and females were decreased in every organ examined (brain, spleen, heart, kidney, liver, adrenal, lung, thyroid, pituitary, thymus, ovaries, uterus, testes, and prostate). The reductions were more prominent (i.e., statistically significant) in males than in females. Significant reductions in absolute organ weights or decreasing trends in absolute organ weights were not observed in low- and mid-dose animals (except for significantly decreased absolute spleen weight in mid-dose males).

Relative organ (to body) weights were significantly elevated in high-dose males and females. Relative organ weights of the spleen and prostate in males and the thymus in females were elevated, but not significantly. No trend was evident in low- and mid-dose animals. The reductions in absolute organ weights and increases in relative organ weights are likely to be secondary to reduced body weight gain after treatment with the test material.

(c) Microscopic Examination

Selected histopathology results are presented in Table 6. Microscopic changes related to treatment were seen in the brain, spinal cord, sciatic nerve, optic nerve, and skeletal muscle of high-dose males and females only (except for degeneration of the optic nerve and brain lesions observed in two mid-dose males). Brain and spinal cord lesions consisted of white matter degeneration and vacuolation of the cytoplasm of neurons. In the spinal cord (ventro-medial and lateral white matter), very slight white matter degeneration was characterized by vacuolation of white matter associated with myelin or cellular debris, macrophages, and pyknotic nuclei within the vacuoles. Very slight vacuolation of the cytoplasm of neurons was most evident in the ventral horn of the spinal cord and in the reticular formation of the brain stem at the level of the cerebellar peduncles. Sciatic and optic nerve lesions were slight, and resembled nervous system lesions consisting of scattered vacuoles. Atrophied skeletal muscle (consistent with gross findings) and trabecular bone were also observed, which may have resulted from the neurogenic atrophy. Necropsy of the nine high-dose male rats sacrificed moribund during the study revealed similar histopathology.

B. DISCUSSION

Despite numerous deficiencies in study reporting and data presentation, this study is rated Core Minimum and satisfies the minimum guideline requirements (82-1) for a subchronic oral toxicity study in rodents. Environmental conditions during the study, the acclimation period, and the method for assigning animals to study groups should be described in greater detail. Statistical analyses should be provided by the study author in the study report. However, since a clear NOEL/LOEL can be determined from these data, this study is considered acceptable for regulatory purposes.

Target dosage levels for the 90-day study were chosen based on the results of a 3-week dietary range-finding study, in which rats received 0, 0.5, 2.5, 5, or 10-20 mg/kg/day (dosage increased at study week 2) in the diet for two weeks. The LOEL for the range-finding study was 10-20 mg/kg/day due to significantly decreased leukocyte, lymphocyte, and neutrophil counts in females, decreased food consumption, and decreased body weight gain. In the 90-day study, the absolute values for the white blood cell parameters were slightly decreased in males, but no trend was observed in females. Dose selection for the 90-day study was appropriate based on the range-finding study, although the data indicated a relatively steep time-dose response curve with continued dosing.

The NOEL for the 90-day study was 2.5 mg/kg/day. The LOEL was 5 mg/kg/day for both sexes. For risk assessment purposes, the high-dose level of 5 mg/kg/day was chosen as the LOEL instead of the time-weighted average high-dose level (6.7 and 7.1 mg/kg/day for males and females,

respectively), because the onset of symptoms (body weight gain, tremors) was very rapid (i.e., not subchronic) at 12.5 mg/kg/day. Because effects were great after one week at 12.5 mg/kg/day, it is likely that 8 mg/kg/day would have produced effects after a few weeks of dosing; therefore, 5 mg/kg/day could represent a subchronic LEL. In addition, this compound may have a long half-life and bioaccumulate since there appeared to be a steep time-dose response (e.g., the incidence of hind limb splay increased with time). The lowest administered high-dose level in this case may therefore represent a more appropriate LOEL.

Treatment-related changes in high-dose animals included moribundity and death, by unscheduled sacrifice, of 9 males. Gross examination at necropsy revealed emaciation, decreased muscle mass, and histological changes in the brain, spinal cord, sciatic and optic nerves, and skeletal muscle. Similar neurotoxic effects have been seen in other species treated with deoxy avermectin. Emaciation, tremors, splaying of hindlimbs, and urogenital staining were seen in both sexes. Body weight and/or food consumption were significantly decreased in both sexes.

Changes that may not have been directly related to treatment included slight but statistically significant increased erythrocytes, hemoglobin, and hematocrit levels in males, and statistically significant ($p \leq 0.01$) decreased glucose and creatinine in both sexes of the high-dose groups. High-dose males also had significantly decreased urine volume and increased urine specific gravity. These changes were probably secondary to generally poor condition, emaciation, decreased food consumption, and possibly dehydration in the high-dose animals .

12

TABLE 1. Incidence/Week of Selected Clinical Observations in High-dose Rats Given Diets of Deoxy Avermectin for 13 Weeks[a,b]

Effect at high dose[c] (12.5/8/5 mg/kg/day)	Incidence[d]/Week at Test Week: 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males														
No. Animals Observed	20	20	20	18	18	18	18	14	14	12	12	11	11	11
Fine tremors	40 (20)	100 (20)	75 (18)	37 (13)	83 (18)	89 (18)	88 (18)	70 (14)	61 (14)	45 (12)	26 (10)	8 (6)	4 (3)	3 (3)
Splaying/limited use of hindlimbs	0	0	0	0	0	0	6 (4)	0	2 (2)	0	3 (2)	10 (2)	6 (2)	2 (2)
Urine staining	0	2	5	0	2 (2)	6 (2)	9 (2)	8 (4)	16 (4)	15 (2)	6 (2)	10 (2)	6 (2)	2 (2)
Females														
No. Animals Observed	20	20	20	20	20	18	18	18	18	18	18	18	18	18
Fine tremors	20 (20)	100 (20)	92 (20)	41 (18)	87 (20)	86 (18)	90 (18)	90 (18)	83 (18)	56 (17)	49 (16)	35 (15)	10 (5)	4 (3)
Splaying/limited use of hindlimbs	0	0	0	0	0	0	0	0	0	0	4 (4)	21 (5)	15 (5)	6 (5)
Urine staining	0	0	0	0	0	0	0	0	0	6 (3)	5 (1)	5 (1)	3 (1)	2 (1)

[a] Data extracted from Study No. TT #88-059-0, Tables A-125 through A-128, pages 384–387.
[b] Incidence data from 20 high-dose rats/sex observed at the start of the study. Two females were found dead week 5. Nine males were sacrificed moribund weeks 3–11. Two females were found dead week 5. Tremors, hindlimb paresis, and urine staining (except for a single incidence in a mid-dose rat) were not observed in any control, low-, or mid-dose animals.
[c] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.
[d] Values represent the total number of times per week that the clinical sign was observed. Values in parentheses indicate the number of animals affected.

TABLE 2. Mean Body Weight (g ± S.D.) at Representative Intervals in Rats given Diets Containing Deoxy Avermectin for 13 Weeks[a,b]

Dietary Level (mg/kg/day)	Mean Body Weight (g ± S.D.) at Week: 1	3	5	7	9	11	13	Average Body Weight Gain
				Males				
0	208±16	315±23	373±28	430±30	465±41	494±40	513±39	360
0.5	211±17 (101)	315±26 (100)	379±35 (102)	442±38 (103)	479±41 (103)	508±47 (103)	528±49 (103)	374
2.5	213±17 (102)	313±30 (99)	372±38 (100)	426±37 (99)	459±47 (99)	489±48 (99)	509±45 (99)	356
12.5/8.0/5.0[c]	173±18** (83)	216±29** (69)	257±40** (69)	263±50** (61)	285±43** (61)	307±42** (62)	329±41** (64)	181
				Females				
0	153±13	202±18	232±22	259±24	276±28	289±32	294±33	173
0.5	145±9 (95)	178±43* (88)	213±18 (92)	237±20* (92)	252±22 (91)	263±23 (91)	268±26 (91)	153
2.5	156±13 (102)	203±21 (100)	233±25 (100)	265±32 (102)	284±34 (103)	296±39 (102)	301±41 (102)	182
12.5/8.0/5.0[c]	125±14** (82)	140±16** (69)	166±24** (72)	178±33** (69)	186±36** (67)	195±39** (67)	204±40** (69)	86

[a] Data extracted from Study No. TT #88-059-0, Tables A-1, A-2, A-15, and A-16, pages 57–72. Statistical analyses (Scheffe's test and ANOVA) performed by the reviewers.
[b] Numbers in parentheses indicate percentage of control value.
[c] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.

TABLE 3. Mean Food Consumption (g/animal/day) at Representative Intervals in Rats given Diets Containing Deoxy Avermectin for 13 Weeks[a,b]

Dietary Level(mg/kg/day)	Mean Food Consumption (g/animal/day ± S.D.) at Week: 1	3	5	7	9	11	13
				Males			
0	22.0±1.9	27.2±2.4	25.7±2.8	25.8±2.6	25.8±4.7	28.7±2.7	27.2±2.5
0.5	22.4±2.2 (102)	27.2±3.5 (100)	26.9±2.7 (105)	26.8±3.2 (104)	27.3±2.6 (106)	28.5±2.4 (99)	28.9±2.8 (106)
2.5	22.9±2.2 (104)	26.4±4.3 (97)	25.3±4.7 (98)	25.1±3.2 (97)	25.8±4.3 (100)	28.2±2.9 (98)	28.4±2.9 (104)
12.5/8.0/5.0[c]	18.9±2.2** (86)	21.2±3.1** (78)	21.7±3.8** (84)	18.5±4.4** (72)	19.3±3.5** (75)	22.5±3.2** (78)	24.6±2.7 (90)
				Females			
0	16.8±1.6	18.7±1.8	18.7±2.1	18.9±2.0	18.5±2.0	19.1±2.4	19.5±2.5
0.5	16.0±1.2 (95)	17.6±1.8 (94)	17.4±1.7 (93)	17.0±2.2 (90)	18.0±1.8 (97)	18.2±1.7 (95)	18.9±2.3 (97)
2.5	17.4±1.8 (104)	19.4±2.8 (104)	19.3±2.6 (103)	19.6±3.6 (104)	19.5±2.7 (105)	19.5±3.0 (102)	20.6±2.7 (106)
12.5/8.0/5.0[c]	13.9±2.3** (83)	14.7±2.0** (79)	16.6±2.6* (89)	15.3±3.5** (81)	15.5±2.8** (84)	16.2±2.6** (85)	17.5±2.5* (90)

[a] Data extracted from Study No. TT #88-059-0, Tables A-17 and A-18, pages 73-88. Statistical analyses (Scheffe's test and ANOVA) performed by the reviewers.
[b] Numbers in parentheses indicate percentage of control value.
[c] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.

TABLE 5. Incidence of Treatment-related Gross Changes in Rats Given Diets of Deoxy Avermectin for 13 Weeks[a]

Effect	Dose (mg/kg/day)			
	0	0.5	2.5	12.5/8.0/5.0[b]
		Males		
Emaciation	0	0	0	9
Skeletal muscle atrophy	0	0	0	11
		Females		
Emaciation	0	0	0	2
Skeletal muscle atrophy	0	0	0	15

[a] Data extracted from Study No. TT #88-059-0, Table B-11 and Table B-12.
[b] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.
20 rats /sex / dose examined

TABLE 6. Incidence of Histopathologic Changes in Rats Given Diets of Deoxy Avermectin for 13 Weeks[a]

Effect	Dose (mg/kg/day)							
	0		0.5		2.5		12.5/8.0/5.0[b]	
	Male	Female	Male	Female	Male	Female	Male	Female
Brain								
Neuronal vacuolation	0	0	0	0	2	0	15	16
Spinal cord								
Neuronal vacuolation	0	0	0	0	0	0	20	18
Degeneration	0	0	0	0	0	0	10	5
Sciatic nerve								
Degneration	0	0	0	0	0	0	17	18
Optic nerve								
Degeneration	0	0	0	0	0	1	1	0
Skeletal muscle								
Atrophy	0	0	0	0	0	0	20	20
Trabecular bone atrophy	0	0	0	0	0	0	17	9

20 rats/sex/dose examined.
[a] Data extracted from Study No. TT #88-059-0, page 402 and Tables B-3 and B-11 through B-13.
[b] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.

APPENDIX B: 3-week Dietary Range-finding Study in Rats

Study No. TT #88-046-0
MRID #427436-20

In this 3-week range-finding study, male and female Sprague-Dawley CRL:CD(SD) BR rats (10/sex/dose) received dietary levels of 0, 5, 25, 50, or 100 ppm (0, 0.5, 2.5, 5, or 10 mg/kg/day) for two weeks. The high dose was increased from 10 to 20 mg/kg/day on day 8 of dosing. Rats were approximately 46 days old and weighed 140-283 g at initiation of dosing.

Slight toxicity was observed during the first week. Decreased average weight gain compared to controls was observed in high-dose males (15% vs 23%) and females (8% vs 11%). Leukocyte, lymphocyte, and neutrophil counts were slightly but significantly lower than control values in females fed 5 mg/kg/day and above.

At the start of week 2 (day 8 of dosing) the high dose was increased from 10 to 20 mg/kg/day. The study authors reported treatment-related effects at 20 mg/kg/day that included decreased food consumption (20 and 33% compared to controls, both sexes) and weight loss (10% compared to week 1, both sexes). Neurotoxicity was also observed and included fine tremors, decreased activity, salivation, and urine staining. One high-dose male was found dead week 3. The reviewers could not confirm these findings because physical observations, mortality, and necropsy findings were not provided in the study report.

Treatment-related effects on hematology parameters included slightly increased erythrocytes, hemoglobin concentration, and hematocrit (10 to 18%, both sexes) in high-dose (20 mg/kg/day, week 2) rats compared to controls. No treatment-related effects were seen in the urine analysis. Serum chemistry tests revealed slight decreases in blood levels of chloride, creatine, and alkaline phosphatase, and increased protein and albumin (both sexes).

Based on the observations from this range-finding study, the NOEL for acute oral toxicity was 5 mg/kg/day. The LOEL was 10-20 mg/kg/day based on significantly decreased leukocyte, lymphocyte, and neutrophil counts in females. Neurotoxicity was reported by the study authors at dose levels of 20 mg/kg/day but could not be confirmed by the reviewer.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS/HED/TB1

TOX ONELINERS

ToxChem No. [Not Available] — MK-0243 **Dated: 02/23/94**

CITATION	MATERIAL	MRID #	RESULTS	TOX. CAT.	COREGRADE
Developmental Tox./ Rat Merck Research Labs. Study #618-244-TOX33 (TT# 89-715-0) Dec. 22, 1992	MK-0243 Benzoate Salt 94.2% a.i. Lot. # 656,748- C 35W002	427436-32 42743631R	MK-0243 was administered orally by gavage to 25 pregnant Sprague-Dawley Crl:CD (SD) BR rats per group at 0, 2, 4, or 8 mg/kg/d during gestation days 6 through 19. Maternal NOEL 2 mg/kg/d LOEL 4 mg/kg/d based on a sig. trend towards decreased body weight gain during the dosing period. Developmental NOEL 4 mg/kg/d LOEL 8 mg/kg/d based on altered growth and an increased incidence of supernumerary rib. CORE CLASSIFICATION: Minimum	N/A	Minimum

APPENDIX A: Additional Statistical Analyses Performed by the Reviewers

TABLE A-1. Selected Hematology Values from Rats given Diets Containing Deoxy Avermectin for 13 Weeks[a]

Parameter		Dietary Level (mg/kg/day) 0	0.5	2.5	12.5/8.0/5.0[b]
			Males		
Erythrocytes (million/mm^3)	week 5	7.57±0.34	7.65±0.39	7.57±0.23	7.95±0.33*
	8	7.77±0.34	8.03±0.30	7.93±0.27	8.40±0.31**
	12	8.16±0.30	8.40±0.36	8.26±0.54	8.5±0.33
Hemoglobin (g/100 mL)	week 5	15.6±0.7	15.4±0.7	15.4±0.5	16.1±0.5**
	8	15.5±0.7	15.6±0.5	15.6±0.5	16.3±0.5**
	12	15.9±0.6	15.9±0.6	15.8±0.8	15.9±0.5
Hematocrit (%)	week 5	43±2	43±2	43±2	45±1**
	8	42±2	43±1	43±2	45±2**
	12	43±2	43±2	43±2	44±2
			Females		
Erythrocytes (million/mm^3)	week 5	7.39±0.36	7.31±0.34	7.40±0.35	7.54±0.27
	8	7.19±0.33	7.18±0.36	7.27±0.26	7.41±0.27
	12	7.59±0.23	7.58±0.34	7.54±0.31	7.66±0.35
Hemoglobin (g/100 mL)	week 5	15.3±0.5	15.1±0.4	15.1±0.5	15.2±0.4
	8	15.0±0.6	15.1±0.4	15.1±0.5	15.5±0.6
	12	15.5±0.4	15.7±0.4	15.5±0.5	15.7±0.6
Hematocrit (%)	week 5	42±1	41±1	41±1	42.1
	8	40±1	41±1	41±1	42±2
	12	42±1	42±1	41±2	43±2

[a] Data extracted from Study No. TT #88-059-0, Tables A-21 through A-26, pages 101-118. Statistical analyses (Scheffe's test and ANOVA) performed by the reviewers.

[b] High dose was decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.

* Significantly different from control value, $p \leq 0.05$.

** Significantly different from control value, $p \leq 0.01$.

2

TABLE A-2. Selected Clinical Chemistry Values from Rats given Diets Containing Deoxy Avermectin for 13 Weeks[a]

Parameter		Dietary Level (mg/kg/day) 0	0.5	2.5	12.5/8.0/5.0[b]
			Males		
Glucose (mg/dL)	week 5	117±16	144±23*	113±22	85±17**
	8	122±13	122±26	114±17	95±30*
	12	144±17	144±23	132±20	102±35**
Total protein (g/dL)	week 5	5.7±0.2	5.7±0.3	5.7±0.2	5.4±0.1*
	8	5.8±0.2	6.0±0.2	6.1±0.2	5.7±0.2
	12	6.0±0.2	6.2±0.2	6.1±0.3	5.8±0.3
Creatinine (mg/dL)	week 5	0.5±0.1	0.5±0.1	0.5±0.1	0.4±0.1*
	8	0.5±0.1	0.5±0.1	0.5±0.1	0.4±0.1**
	12	0.6±0.1	0.6±0.1	0.6±0.1	0.4±0.1**
Blood urea nitrogen (mg/dL)	week 5	13.7±2.4	14.4±1.9	14.2±2.7	14.3±2.1
	8	13.8±1.8	14.1±1.9	13.5±1.6	15.2±4.4
	12	14.6±1.8	15.1±1.8	14.2±1.8	16.0±4.3
A/G ratio	week 5	1.3±0.1	1.3±0.2	1.3±0.1	1.5±0.1
	8	1.2±0.1	1.2±0.1	1.2±0.1	1.3±0.1*
	12	1.1±0.0	1.1±0.1	1.1±0.1	1.2±0.1*
			Females		
Glucose (mg/dL)	week 5	109±28	94±14	109±27	75±18**
	8	133±24	112±17	134±25	102±31**
	12	137±14	133±18	149±20	100±27**
Total protein (g/dL)	week 5	5.7±0.3	5.7±0.3	5.7±0.3	5.6±0.3
	8	5.9±0.2	5.8±0.2	5.9±0.2	5.8±0.3
	12	6.3±0.3	6.3±0.2	6.4±0.3	6.2±0.3
Creatinine (mg/dL)	week 5	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1**
	8	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1**
	12	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1**
Blood urea nitrogen (mg/dL)	week 5	15.4±1.9	15.5±2.1	15.8±2.3	17.3±3.1**
	8	13.7±1.6	14.8±1.7	14.7±2.3	17.2±3.4**
	12	14.3±1.5	15.0±2.4	15.0±2.6	16.0±2.5
A/G ratio	week 5	1.5±0.1	1.5±0.2	1.5±0.2	1.6±0.2
	8	1.3±0.1	1.3±0.1	1.3±0.1	1.4±0.1*
	12	1.4±0.1	1.3±0.1	1.3±0.1	1.4±0.1*

[a] Data extracted from Study No. TT #88-059-0, Tables A-71 through A-78, A-81, and A-82, pages 248-271 and 278- 283. Statistical analyses (Scheffe's test and ANOVA) performed by the reviewers.

[b] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.

* Significantly different from control value, $p \leq 0.05$.

** Significantly different from control value, $p \leq 0.01$.

TABLE A-3. Selected Urinalysis Values from Rats given Diets Containing Deoxy Avermectin for 13 Weeks[a]

Parameter		Dietary Level (mg/kg/day) 0	0.5	2.5	12.5/8.0/5.0[b]
			Males		
Volume (mL)	week 8	24.7±8.5	25.6±10.8	19.6±7.2	9.4±4.5**
	12	23.6±7.2	19.0±8.7	19.8±11.4	10.8±3.5*
Specific gravity	week 8	1.017±0.007	1.017±0.006	1.020±0.007	1.034±0.013**
	12	1.017±0.006	1.023±0.008	1.023±0.011	1.030±0.010*
			Females		
Volume (mL)	week 8	13.7±10.9	13.7±7.4	16.2±4.9	6.8±3.5
	12	12.8±7.0	11.1±6.2	14.7±7.2	6.5±4.8
Specific gravity	week 8	1.022±0.014	1.020±0.020	1.012±0.005	1.035±0.014
	12	1.024±0.010	1.026±0.011	1.021±0.010	1.039±0.018

[a] Data extracted from Study No. TT #88-059-0, Tables A-99, A-100, A-103, and A-104, pages 332-335 and 340-343. Statistical analyses (Scheffe's test and ANOVA) performed by the reviewers.
[b] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.

TABLE A-4. Summary of Absolute (g ± S.D.) and Relative (%) Organ Weights in Female Rats Given Diets of Deoxy Avermectin for 13 Weeks[a]

Organ (female)	Dose (mg/kg/day) 0	0.5	2.5	12.5/8.0/5.0[b]
Body Weight (B.W.)	275±29	248±27	282±38	188±40**
Brain	1.87±0.08	1.86±0.05	1.86±0.09	1.82±0.09**
% B.W.	0.69±0.08	0.76±0.08	0.67±0.09	1.00±0.21**
Spleen	0.51±0.09	0.47±0.08	0.52±0.14	0.42±0.14**
% B.W	0.19±0.02	0.19±0.03	0.18±0.04	0.23±0.06**
% Br.W.	27±5	25±5	28±8	23±7
Heart	1.01±0.11	0.92±0.10	1.00±0.14	0.87±0.15*
% B.W.	0.37±0.03	0.37±0.04	0.36±0.03	0.47±0.06**
% Br.W.	54±7	50±5	54±7	48±8
Kidneys	2.16±0.26	1.98±0.18	2.15±0.24	1.92±0.35**
% B.W.	0.79±0.07	0.8±0.06	0.77±0.07	1.04±0.16**
% Br.W.	116±16	106±10	116±12	106±18
Liver	7.64±0.97	7.35±1.03	8.05±1.17	6.78±1.34**
% B.W.	2.78±0.21	2.96±0.25	2.86±0.20	3.64±0.44**
% Br.W.	409±55	395±54	432±62	373±69
Adrenals	0.071±0.011	0.071±0.011	0.072±0.012	0.064±0.009**
% B.W.	0.026±0.004	0.029±0.006	0.026±0.003	0.035±0.009**
% Br.W.	3.8±0.7	3.8±0.6	3.9±0.7	3.5±0.5
Lungs	1.22±0.14	1.20±0.15	1.31±0.26	1.09±0.19**
% B.W.	0.45±0.04	0.48±0.04	0.47±0.08	0.57±0.09**
% Br.W.	65±7	64±8	71±15	60±11
Thryoid	0.016±0.004	0.016±0.002	0.015±0.002	0.015±0.003
% B.W.	0.0059±0.0011	0.0065±0.0010	0.0055±0.0007	0.0080±0.0022**
% Br.W.	0.88±0.23	0.86±0.12	0.83±0.13	0.80±0.17
Pituitary	0.015±0.002	0.014±0.002	0.015±0.002	0.013±0.002
% B.W.	0.0056±0.0009	0.0057±0.0010	0.0054±0.0007	0.0073±0.0010**
% Br.W.	0.82±0.12	0.75±0.11	0.80±0.12	0.74±0.11
Thymus	0.30±0.08	0.25±0.05	0.27±0.08	0.23±0.05*
% B.W.	0.11±0.03	0.10±0.02	0.10±0.03	0.13±0.03
% Br.W.	16±4	14±3	15±4	13±3
Ovaries	0.080±0.014	0 080±0.011	0.082±0.014	0.074±0.014**
% B.W.	0.029±0.004	0.032±0.005	0.029±0.005	0.040±0.009**
% Br.W.	4.3±0.8	4.3±0.6	4.4±0.8	4.1±0.8
Uterus	0.71±0.20	0.61±0.19	0.54±0.16	0.67±0.29*
% B.W.	0.26±0.07	0.25±0.08	0.19±0.06	0.37±0.20*
% Br.W.	38±11	33±10	29±8	37±16

Br.W. = brain weight

[a] Data extracted from Study No. TT #88-059-0, Table B-1, pages 405–406.

[b] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.

* Significantly different from control value; p≤0.05.

** Significantly different fron control value; p≤0.01.

TABLE A-5. Summary of Absolute (g ± S.D.)and Relative (%) Organ Weights in Male Rats Given Diets of Deoxy Avermectin for 13 Weeks[a]

Organ (male)	Dose (mg/kg/day) 0	0.5	2.5	12.5/8.0/5.0[b]
Body Weight (B.W.)	484±37	496±47	478±42	303±43**
Brain	2.10±0.08	2.09±0.08	2.10±0.08	2.00±0.11*
% B.W.	0.44±0.04	0.42±0.04	0.44±0.03	0.67±0.09**
Spleen	0.77±0.12	0.77±0.10	0.68±0.09*	0.54±0.09**
% B.W	0.16±0.02	0.16±0.02	0.14±0.02**	0.18±0.03**
% Br.W.	37±5	37±5	32±4*	27±4**
Heart	1.53±0.13	1.53±0.13	1.46±0.13	1.21±0.13**
% B.W.	0.32±0.02	0.31±0.02	0.31±0.03	0.41±0.05**
% Br.W.	73±6	73±7	70±6	61±5**
Kidneys	3.70±0.31	3.70±0.40	3.78±0.36	3.18±0.55**
% B.W.	0.77±0.06	0.75±0.06	0.79±0.05	1.06±0.17**
% Br.W.	177±16	177±20	179±15	159±21*
Liver	13.61±1.54	14.04±2.17	13.83±1.70	10.49±1.49**
% B.W.	2.81±0.18	2.82±0.19	2.89±0.20	3.48±0.31**
% Br.W.	650±78	673±106	656±67	524±63**
Adrenals	0.060±0.007	0.061±0.009	0.064±0.011	0.057±0.012**
% B.W.	0.012±0.002	0.013±0.003	0.013±0.002	0.019±0.004**
% Br.W.	2.9±0.4	2.9±0.4	3.0±0.5	2.9±0.5
Lungs	1.66±0.17	1.71±0.17	1.66±0.16	1.40±0.16**
% B.W.	0.34±0.04	0.35±0.03	0.35±0.04	0.47±0.06**
% Br.W.	79±8	82±9	79±7	70±6*
Thryoid	0.021±0.004	0.022±0.003	0.021±0.004	0.018±0.003*
% B.W.	0.0043±0.0008	0.0045±0.0006	0.0044±0.0006	0.0059±0.0012**
% Br.W.	0.99±0.20	1.08±0.15	1.00±0.15	0.88±0.14
Pituitary	0.014±0.002	0.013±0.002	0.014±0.002	0.012±0.002**
% B.W.	0.0028±0.0004	0.0027±0.0004	0.0029±0.0003	0.0039±0.0008**
% Br.W.	0.65±0.07	0.64±0.09	0.66±0.08	0.58±0.09
Thymus	0.30±0.06	0.31±0.10	0.31±0.08	0.24±0.04*
% B.W.	0.06±0.01	0.06±0.02	0.06±0.02	0.08±0.02*
% Br.W.	14±3	15±5	14±4	12±2
Testes	3.37±0.32	3.33±0.30	3.30±0.41	3.21±0.23**
% B.W.	0.70±0.08	0.68±0.09	0.70±0.11	1.08±0.19**
% Br.W.	161±16	160±15	157±20	161±12
Prostate	0.61±0.20	0.61±0.18	0.65±0.20	0.42±0.15
% B.W.	0.13±0.04	0.12±0.03	0.14±0.04	0.14±0.05
% Br.W.	29±9	29±9	31±10	21±7

Br.W. = brain weight

[a] Data extracted from Study No. TT #88-059-0, Table B-2, pages 407–408.

[b] High dose decreased from 12.5 to 8 mg/kg/day in week 3, and from 8 to 5 mg/kg/day in week 9.

* Significantly different from control value, p≤0.05.

** Significantly different from control value, p≤0.01.