FINAL

2/22/1994

DATA EVALUATION REPORT

MK-0244

Study Type: Chronic Oral Toxicity in Dogs

Prepared for:

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Health Effects Division
U.S. Environmental Protection Agency
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DATA EVALUATION REPORT

STUDY TYPE: Chronic oral toxicity in dogs -- Guideline Series 83-1

TEST MATERIAL: MK-0244

TOX. CHEM. NUMBER: New chemical P.C. NUMBER: 122806

SYNONYMS: L-656,748 038W, deoxy avermectin

MRID NUMBER: 427636-24

STUDY NUMBERS: 90-612-0 and 90-612-01
Laboratory Project Identification 618-244-TOX21

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

TESTING FACILITY: Laboratories Merck Sharp & Dohme-Chibret
Centre de Recherche
Riom, France

TITLE OF REPORT: MK-0244. 53-Week Oral Toxicity Study in Dogs

AUTHOR: J-P. Gillet

REPORT ISSUED: Study completed December 18, 1992

QUALITY ASSURANCE: A signed Good Laboratory Practice Compliance Statement, a signed Quality Assurance Statement, and a list of Quality Assurance Inspections were included.

CONCLUSIONS: MK-0244 was administered to beagle dogs (4/sex/dose) via gavage in distilled water for up to 53 weeks at doses of 0, 0.25, 0.5, 0.75, and 1.0 mg/kg/day. Administration of the 0.75-mg/kg/day dose was initiated approximately 4 weeks after the other doses because of extreme toxicity observed at the 1.0-mg/kg/day dose.

NOEL = 0.25 mg/kg/day

LOEL = 0.5 mg/kg/day based on axonal degeneration in the pons, medulla, and peripheral nerves (sciatic, sural, and tibial) in both males and females, clinical signs of neurotoxicity (whole body tremors, stiffness of the hind legs), spinal cord axonal degeneration, and muscle fiber degeneration in females.

At 0.75 mg/kg/day, males were sacrificed after only 7 weeks of exposure. Mydriasis, cellular degeneration in the retina, axonal degeneration in the optic nerve, and decreased body weight gain were observed in both males and females at this dose. Females at this dose showed difficulty getting up, ataxia, and hyperreactivity to touch. Males at this dose and above also showed stiffness of the hind legs, axonal degeneration in the spinal cord, focal neuronal degeneration in the brain, muscle fiber degeneration, and decreased food consumption.

At the highest dose tested, 1.0 mg/kg/day, both males and females were sacrificed after only 3 weeks of exposure. Both males and females at this dose exhibited decreased motor activity, and females showed focal neuronal degeneration in the brain and decreased food consumption.

CORE CLASSIFICATION: Core Minimum. This study satisfies the guideline requirements for a chronic toxicity study in a nonrodent species and is classified as Core Minimum because of the early sacrifices at the 2 highest doses, incomplete histopathology at the 2 lowest doses, incomplete food consumption data, lack of calculation of means for males and females for several parameters, and failure to perform statistical analyses.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: MK-0244

Formula: Not provided

Lot number: 002

Purity: >97%

Physical properties: Not reported

Stability: Not reported

2. Rationale for Dose Selection

No information was provided regarding the rationale for selection of the dietary levels of MK-0244 used in the current study. However, a 13-week feeding study (MRID 427436-22) was conducted in which doses of 0, 0.5, 1.0 and 1.5 were initially tested but were lowered to 0, 0.25, 0.5, and 1.0 mg/kg/day due to excessive clinical signs of toxicity (ataxia, lateral recumbency, mydriasis). The NOEL was determined to be 0.25 mg/kg/day and the LOEL was 0.5 mg/kg/day based on skeletal muscle atrophy and histopathological findings in the brain and spinal cord.

3. Test Article Analyses for Purity and Stability

All analyses of the test material were conducted using HPLC. The purity of the test material was verified by the testing facility prior to the initiation of the study and then at approximately 6 and 12 months. The purity, as determined by the testing facility, ranged from 97.15% to 98.39%.

The test material was prepared for gavage dosing (5 mL/kg) by mixing appropriate amounts of MK-0244 with distilled water. The concentrations used for the 0.25-, 0.5-, 0.75-, and 1.0-mg/kg doses were 0.057, 0.114, 0.171, and 0.224 mg/mL, respectively. The benzoate salt was used; therefore, a factor of 1.14 was used in the dosage calculations. Controls received gavage doses of distilled water. Fresh solutions were prepared daily.

Stability of the lowest and highest concentration solutions for 24 hours was measured during the first week of dosing. No loss of test material was detected.

The actual concentration of the test material in the gavage solutions was measured at the start of dosing and then approximately every 3 months thereafter. The average measured concentrations at each test level were as follows:

Table 1. Measured Concentration at Each Dose Level

Dosage Group (mg/kg/day)	Nominal Concentration (mg/mL)	Measured Concentration[a] (mg/mL)
0.25	0.057	0.052 ± 0.001
0.50	0.114	0.105 ± 0.004
0.75	0.171	0.169 ± 0.007
1.00	0.228	0.211[b]

[a]Mean ± S.D., data from Appendix I of the study report, calculated by reviewer
[b]This value was determined only once

Mean test concentrations were within 10% of the target concentrations.

4. Animals

Beagle dogs (20 males and 20 females) were received from Marshall Farms, North Rose, NY. No information was provided regarding whether the dogs used for the 0.75-mg/kg dose (4 males and 4 females; study #90-612-1) were received at the same time as the dogs used in the main study (#90-612-0). The dogs were caged individually in stainless steel cages. The animal room was operated on a 12-hour light/dark cycle, temperature was maintained at approximately 19°C,

and relative humidity ranged from 30% to 70%. No information was provided regarding the existence of an acclimation period.

Dogs were randomly assigned to study groups (4/sex/dose) as shown below:

Table 2. Study Design

Exposure Level (mg/kg/day)	Number of Animals	
	Males	Females
0	4	4
0.25	4	4
0.50	4	4
0.75	4	4
1.00	4	4

Test material was administered by gavage 7 days/week. At the time of the first exposure to test material, the dogs were between 8 and 11 months old, and males and females ranged in weight from 8.7 to 12.2 kg and from 6.5 to 12.1 kg, respectively. The Subdivision F guidelines specify that dogs be no more than 9 months of age when dosing is undertaken, but this is a minor deficiency and should not have affected the study outcome. The dogs were uniquely identified through the use of labelled collars.

Water was provided ad libitum. Three hundred grams of pelleted feed (UAR Lab Chow) were provided to most dogs each day. In an attempt to reverse the body weight losses of 4 dogs (2 males at 0.25 mg/kg/day, 1 male at 0.5 mg/kg/day, and 1 female at 0.75 mg/kg/day) the male dogs received up to 600 g of feed each day starting at week 46 and continuing until the end of the study and the female received this amount from week 43 to week 50. In addition, 3 dogs with decreased food consumption received soft food for 1-2-week periods in an effort to increase food consumption. A female at 1.0 mg/kg/day received soft food during weeks 3 and 4, a female at 0.5 mg/kg/day received soft food during weeks 32 and 33, and a female at 0.25 mg/kg/day received soft food during week 30.

5. Statistical Analyses

Data were not analyzed statistically.

6. General Observations

(a) Mortality/moribundity/survival

Animals were observed three times daily (once just prior to dosing, once just after dosing, and once 4-6 hours after dosing) for mortality/moribundity.

Results - All the dogs in 1-mg/kg/day group were sacrificed after 19 doses (and a 4-day recovery period) because of excessive neurotoxicity, decreased food consumption, and weight loss. In addition, all males at 0.75 mg/kg/day were sacrificed after 49 doses and a 1-day recovery period because of excessive weight loss and neurotoxicity. No deaths or other unscheduled sacrifices occurred in other groups.

(b) Clinical observations

Animals were observed three times daily (once just prior to dosing, once just after dosing, and once 4-6 hours after dosing) for overt adverse clinical signs. Less-detailed physical examinations were conducted on weekends and holidays. In addition, neurological testing for cranial and spinal nerve reflexes, gait, and postural reactions were conducted as follows:

Controls	(examined, but dates not specified)
0.25 mg/kg/day	(at weeks 26 and 52)
0.50 mg/kg/day	(at weeks 26 and 52)
0.75 mg/kg/day	(at weeks 4 and 7 for males and weeks 4, 8, 22, 48, and 52 for females)
1.0 mg/kg/day	(at week 3)

Results - As seen in Tables 3a and 3b, by 3 weeks of exposure, daily physical examination revealed that most dogs at 1.0 mg/kg/day exhibited mydriasis, decreased motor activity, and fine whole body tremors. Mydriasis was the earliest symptom recorded, and was observed in 2 females and 1 male at this dose by week 2 of exposure. By the end of 7 weeks of exposure, dogs at 0.75 mg/kg/day showed mydriasis and fine whole body tremors. Males at this dose also showed stiffness of the hind legs. Tremors were the earliest symptom observed at this dose, and were observed in 2 males and 1 female as early as 5 weeks of exposure. By week 26 (week 22 at 0.75 mg/kg/day), 1 female at 0.50 mg/kg/day showed fine whole body tremors and females at 0.75 mg/kg/day continued to show fine-to-moderate whole body tremors and mydriasis. In addition, females at this dose showed stiffness of the hind legs, and neurological examination revealed difficulty getting up. The onset of the symptoms in the female at 0.5 mg/kg/day was at week 15. By the end of the study, the female at 0.5 mg/kg/day showed fine whole body tremors and stiffness of the hind legs. The females at 0.75 mg/kg/day showed fine-to-moderate whole body tremors, stiffness of the hind legs, mydriasis, difficulty getting up, ataxia, and hyperreactivity to touch.

(c) Body weights/food consumption/feed efficiency/test article intake

Body weights--Individual body weights were determined pretest, once during week 1, and then twice weekly throughout the study.

Results - By the end of the third week of exposure, weight losses were observed in both males and females at 1.0 mg/kg/day and in

Table 3a. Incidence of Clinical Signs in Male Dogs Ingesting MK-0244 in the Diet for up to 1 Year[a]

	Incidence by Dietary Level (mg/kg/day)[b]				
Parameter	0	0.25	0.5	0.75	1.0
Whole body tremors					
3wk	0/4	0/4	0/4	0/4	4/4
7wk	0/4	0/4	0/4	4/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-
Mydriasis					
3wk	0/4	0/4	0/4	0/4	3/4
7wk	0/4	0/4	0/4	1/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-
Decreased motor activity					
3wk	0/4	0/4	0/4	0/4	2/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-
Stiff hind legs					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	2/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-
Ataxia					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-
Difficulty getting up					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-
Hyperreactivity to touch					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	-	-
52wk	0/4	0/4	0/4	-	-

[a] Data extracted from Study #90-612-0 and Study #90-612-01, Appendix VI
[b] The numerators represent the number of animals affected at each time point. Dashes indicate that the animals died or were sacrificed prior to this reading.

7

Table 3b. Incidence of Clinical Signs in Female Dogs Ingesting MK-0244 in the Diet for up to 1 Year[a]

	Incidence by Dietary Level (mg/kg/day)[b]				
Parameter	0	0.25	0.5	0.75	1.0
Whole body tremors					
3wk	0/4	0/4	0/4	0/4	4/4
7wk	0/4	0/4	0/4	2/4	-
26wk	0/4	0/4	1/4	2/4	-
52wk	0/4	0/4	1/4	3/4	-
Mydriasis					
3wk	0/4	0/4	0/4	0/4	4/4
7wk	0/4	0/4	0/4	1/4	-
26wk	0/4	0/4	0/4	1/4	-
52wk	0/4	0/4	0/4	1/4	-
Decreased motor activity					
3wk	0/4	0/4	0/4	0/4	4/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	2/4	-
52wk	0/4	0/4	0/4	0/4	-
Stiff hind legs					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	2/4	-
52wk	0/4	0/4	1/4	2/4	-
Ataxia					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	0/4	-
52wk	0/4	0/4	0/4	1/4	-
Difficulty getting up					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	2/4	-
52wk	0/4	0/4	0/4	2/4	-
Hyperreactivity to touch					
3wk	0/4	0/4	0/4	0/4	0/4
7wk	0/4	0/4	0/4	0/4	-
26wk	0/4	0/4	0/4	0/4	-
52wk	0/4	0/4	0/4	1/4	-

[a] Data extracted from Study #90-612-0 and Study #90-612-01, Appendix VI
[b] The numerators represent the number of animals affected at each time point. Dashes indicate that the animals died or were sacrificed prior to this reading.

8

males at 0.75 mg/kg/day (Tables 4 and 5). With continued exposure, the males at 0.75 mg/kg/day continued to lose weight, and by 7 weeks of exposure, the body weights and body weight gain of this group were significantly lower than the controls. (mean body weight 18% less than controls) Although substantial weight losses were not observed in females at 0.75 mg/kg/day, the body weight gain of this group lagged behind that of controls for most of the study. The effect on weight gain in females at 0.75 mg/kg/day was not statistically significant. (35% less than controls at 52 wks)

Food consumption--Individual food consumption values were determined 4 times/week.

Results - Average weekly food consumption was significantly decreased in females at 1.0 mg/kg/day at week 3 (38% of control) and in males at 0.75 mg/kg/day at week 7 (80% of control). Other than these changes, food consumption data showed no substantive differences between groups. Most male dogs ate their entire ration each day. Females generally ate between 80% and 100% of their ration each day. It should be noted, however, that during the last 2.5 months of the study, when the dogs with body weight losses received extra rations (see Section 4, above), the food consumption of these dogs was not recorded. Also, food intake was not recorded for one female at 0.5 mg/kg/day that received soft food (because of low food consumption) during weeks 32 and 33. Thus, food intake data for these periods are incomplete. Because the dogs that received soft food or extra rations were not exclusively high-dose dogs (2 males and 1 female at 0.25 mg/kg/day, 1 male and 1 female at 0.5 mg/kg/day, and 1 female at 0.75 mg/kg/day), these omissions should not affect the conclusions regarding food intake.

Feed efficiency--Feed efficiency was not determined in this study.

(d) Ophthalmoscopic examinations

Eye examinations were conducted by indirect ophthalmoscopy and slit lamp prior to the first exposure. Tropicamide was used to dilate the pupils. Dogs in the control group and the 0.25- and 0.5-mg/kg/day groups were also examined after 11, 25, and 51 weeks of exposure. Dogs in the 0.75-mg/kg/day group were examined after 7, 21, 47, and 52 weeks of exposure.

Results - No treatment-related effects on the appearance of the eyes were reported. However, there was no letter from a D.V.M. substantiating that this exam was performed. Based on histopathological lesions observed in the retina (see below) it might be expected that a detailed ophthalmogical examination would show some degeneration in the retina.

Table 4. Summary of Mean Body Weights (kg ± S.D.) of Dogs Ingesting MK-0244 in the Diet for up to 1 Year[a,b]

	Body Weight by Dietary Level (mg/kg/day)[c]				
Week	0	0.25	0.5	0.75	1.0
			Males		
Pretest	10.2 ± 1.2	10.6 ± 1.2 (104)	10.5 ± 0.8 (103)	10.1 ± 0.4 (99)	10.4 ± 1.2 (102)
3	10.3 ± 1.2	10.7 ± 1.3 (104)	10.6 ± 0.8 (103)	9.6 ± 0.1 (93)	10.1 ± 1.2 (98)
7	10.6 ± 1.2	10.8 ± 1.3 (102)	10.8 ± 0.6 (102)	8.7 ± 1.0* (82)	-
13	10.5 ± 1.2	10.7 ± 1.4 (102)	10.8 ± 0.6 (103)	-	-
26	10.9 ± 1.4	10.6 ± 1.6 (97)	11.0 ± 0.9 (101)	-	-
39	11.0 ± 1.6	10.6 ± 1.8 (96)	11.2 ± 1.6 (102)	-	-
52	11.0 ± 2.0	12.3 ± 0.9 (112)	12.2 ± 0.8 (111)	-	-
			Females		
Pretest	9.0 ± 2.2	8.3 ± 1.4 (92)	8.4 ± 1.7 (93)	9.1 ± 0.6 (101)	8.3 ± 1.3 (92)
3	9.1 ± 2.4	8.5 ± 1.4 (93)	8.6 ± 1.6 (95)	9.0 ± 0.7 (99)	7.5 ± 0.7* (82)
7	9.4 ± 2.7	8.7 ± 1.2 (93)	8.8 ± 1.2 (94)	9.1 ± 0.8 (97)	-
13	9.5 ± 3.0	8.8 ± 1.2 (93)	8.8 ± 1.0 (93)	9.1 ± 1.0 (96)	-
26	10.3 ± 3.4	9.3 ± 1.3 (90)	9.2 ± 1.1 (89)	9.5 ± 1.4 (92)	-
39	10.7 ± 3.3	9.7 ± 1.0 (91)	9.9 ± 0.6 (93)	9.5 ± 1.5 (89)	-
52	11.4 ± 3.8	10.4 ± 1.0 (91)	10.3 ± 0.8 (90)	10.7 ± 1.5 (94)	-

[a] Data extracted from Study #90-612-0 and Study #90-612-01, Table A-1; means calculated by reviewers
[b] Numbers in parentheses represent percent control
[c] Dashes indicate that the animals had died or were sacrificed prior to this reading.

* Significantly different from control; $p \leq 0.05$ by analysis of variance and Scheffe's test, performed by the reviewers

Table 5. Average Body Weight Gain Data (kg ± S.D) for Dogs Ingesting MK-0244 in the Diet for up to 1 Year[a]

	Body Weight Gain by Dietary Level (mg/kg/d)[b]				
Interval	0	0.25	0.5	0.75	1.0
			Males		
0-3	0.10 ± 0.29	0.00 ± 0.24	0.10 ± 0.18	-0.45 ± 0.39	-0.23 ± 0.25
4-7	0.38 ± 0.28	0.25 ± 0.42	0.30 ± 0.32	-1.43 ± 1.18*	-
8-13	0.28 ± 0.55	0.13 ± 0.57	0.28 ± 0.71	-	-
14-26	0.70 ± 0.90	0.05 ± 1.06	0.53 ± 1.11	-	-
27-39	0.80 ± 1.01	0.05 ± 1.35	0.75 ± 1.72	-	-
40-52	0.83 ± 1.25	1.75 ± 0.71	1.73 ± 1.52	-	-
			Females		
0-3	0.13 ± 0.28	0.15 ± 0.13	0.20 ± 0.22	-0.10 ± 0.14	-0.78 ± 0.68
4-7	0.45 ± 0.51	0.40 ± 0.24	0.38 ± 0.62	0.00 ± 0.28	-
8-13	0.48 ± 0.82	0.45 ± 0.31	0.43 ± 0.90	0.03 ± 0.57	-
14-26	1.33 ± 1.20	0.95 ± 0.56	0.80 ± 1.06	0.38 ± 0.95	-
27-39	1.70 ± 1.17	1.38 ± 0.76	1.50 ± 1.68	0.65 ± 1.48	-
40-52	2.45 ± 1.58	2.03 ± 0.72	1.88 ± 1.63	1.60 ± 0.98	-

[a] Data extracted from Study #90-612-0 and Study #90-612-01, Table A-1; means calculated by reviewers
[b] Dashes indicate that the animals had died or were sacrificed prior to this reading.
* Significantly different from control; $p \leq 0.05$ by analysis of variance and Scheffe's test performed by the reviewers

7. Clinical Pathology

Hematological, blood chemistry, and urine analyses were performed on all dogs prior to initiation of dosing. Dogs at 0.25 and 0.5 mg/kg/day were also examined after 13, 25, and 52 weeks of exposure. Dogs at 0.75 mg/kg/day were examined after 7, 9, 21, 48, and 52 weeks of exposure. Control dogs were examined at weeks 11 (corresponds to week 7 at 0.75 mg/kg/day), 13, 25, and 52. Blood samples were obtained from the jugular vein, and urine was collected overnight in the animals' cages. The study report did not indicate whether animals were fasted prior to bleeding.

(a) Hematology

The parameters marked with an "X" were examined.

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

* Recommended by Subdivision F (November 1984) Guidelines

Results - No treatment-related effects were observed.

(b) Blood (clinical) chemistry

Blood chemistry analyses included the parameters marked below with an "X."

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

Enzymes
X Lactate dehydrogenase
X Creatine phosphokinase*
X Alkaline phosphatase (ALP)
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*

Other
X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
X Total cholesterol*
X Total protein*
X Glucose*
X Total bilirubin*
X Triglycerides

* Recommended by Subdivision F (November 1984) Guidelines

Results - No treatment-related effects were observed.

12

(c) Urinalysis

Urinalysis included the parameters marked below with an "X."

X Appearance*	X Sediment (microscopic)*	X Bilirubin
X Volume*	X Protein*	X Blood*
X Specific gravity*	X Glucose*	X Urobilinogen
X pH	X Ketones*	

* Recommended by Subdivision F (November 1984) Guidelines

Results - Urinalysis showed increases in bilirubin (275% of control) and urobilinogen (200% of control) content of the urine after 7 weeks of exposure in males at 0.75 mg/kg/day (Table 6). However, in the absence of any liver pathology (see below), the biological significance of this finding is unclear.

8. Sacrifice and Pathology

All dogs sacrificed in extremis or at study termination by exsanguination under anesthesia received a complete gross examination. Tissues that are marked with an "X" below were examined histologically in controls, in males at 0.5 mg/kg/day, and in all dogs at 0.75 and 1.0 mg/kg/day. In addition, skeletal muscle, brain, spinal cord, nerves, and eye (including optic nerve) were examined in all dogs. All tissues were preserved in neutral buffered 10% formalin solution except for the testes and epididymides, which were fixed in Bouin's fixative, and the eyes, which were fixed in Zenker's solution prior to selection of sections for staining with hematoxylin and eosin. Organs that are marked with a "XX" were also weighed at necropsy for all animals. The heart, spleen, lungs, pituitary, prostate, ovaries, and uterus were also weighed in animals that were sacrificed in extremis.

Table 6. Mean Severity Scores of Bilirubin and Urobilinogen Content of Urine from Dogs Ingesting MK-0244 in the Diet for up to 1 Year[a,b]

Parameter	Mean Severity Score by Dietary Level (mg/kg/day)[c]			
	0	0.25	0.5	0.75
		Males		
Bilirubin				
Pretest	1.25	1.50	1.00	0.75
Week 7/11	1.00	ND	ND	2.75
Week 9/13	1.00	1.00	0.50	-
Week 21/25	1.50	1.75	1.00	-
Week 48/52	1.00	1.75	0.75	-
Urobilinogen				
Pretest	1.25	1.25	0.50	0.75
Week 7/11	1.25	ND	ND	2.50
Week 9/13	0.75	0.50	0.00	-
Week 21/25	1.25	1.50	0.50	-
Week 48/52	1.50	1.50	0.75	-
		Females		
Bilirubin				
Pretest	1.00	0.75	1.00	0.50
Week 7/11	0.25	ND	ND	0.50
Week 9/13	0.00	0.25	0.25	0.50
Week 21/25	0.50	0.00	0.25	0.50
Week 48/52	0.25	0.25	0.00	0.00
Urobilinogen				
Pretest	0.50	0.50	0.75	0.75
Week 7/11	0.00	ND	ND	0.50
Week 9/13	0.00	0.00	0.00	0.25
Week 21/25	0.25	0.00	0.00	0.75
Week 48/52	0.50	0.25	0.50	0.50

[a] Data extracted from Study #90-612-0 and Study #90-612-01, Tables A-53 and A-56; mean scores calculated by reviewers

[b] Severity scores defined as: 1 = 1 mg/dL, 2 = 2 mg/dL, and 3 = 3 mg/dL for bilirubin; 1 = 2 mg/dL, 2 = 4 mg/dL, 3 = 8 mg/dL, and 4 = 12 mg/dL for urobilinogen

[c] Dashes indicate that the animals died or were sacrificed prior to this reading.

ND = Not done

14

Digestive System	Cardiovascular/Hematologic	Neurologic
X Pancreas*	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Peripheral nerve* (sciatic, tibial, and (sural nerves)
X Esophagus*	X Bone marrow*	X Pituitary*
X Stomach*	X Lymph nodes*	X Eyes/optic nerve*
X Duodenum*	X Spleen*	X Spinal cord* (three levels)
X Jejunum*	X Thymus*	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*		X Parathyroids*
X Rectum*	XX Kidneys*	X Mammary gland*
XX Liver*	X Urinary bladder*	XX Thyroids*
	XX Testes*	XX Adrenals*
Respiratory	XX Epididymides*	
	X Prostate*	
X Trachea*	Vagina*	
X Lungs*	X Ovaries*	
	X Uterus*	
Other	Seminal vesicles*	
X Gallbladder*	X Oviducts	
X Skin*		
X Bone (femur)*		
X Skeletal muscle (thigh)*		
X All gross lesions and masses*		

* Recommended by Subdivision F (November 1984) Guidelines

Note: The Subdivision F Guidelines recommend full histopathology in all nonrodents. However, only target organs (skeletal muscle, brain, spinal cord, nerve, eye) and tissues with gross lesions were examined at 0.25 mg/kg/day in both sexes and at 0.5 mg/kg/day in females.

(a) Macroscopic examination

No treatment-related effects were observed at gross necropsy.

(b) Organ weights

No treatment-related effects on absolute or relative organ weights were observed.

(c) Microscopic examination

Histopathologic analysis showed treatment-related changes in nervous system tissues and in skeletal muscle (Table 7). At 0.5 mg/kg/day and above, both males and females showed axonal degeneration in the brain (pons and medulla) and nerve (sciatic, sural, and tibial). Axonal degeneration in the spinal cord was observed in males at 0.75 mg/kg/day and above and in females at 0.5 gm/kg/day and above. Focal neuronal degeneration in the brain was observed in males at 0.75 mg/kg/day and in both sexes

Table 7. Incidence of Histopathology in Dogs Ingesting MK-0244 in the Diet for 1 Year[a,b]

Parameter	Histopathology Incidence by Dietary Level (mg/kg/day)				
	0	0.25	0.5	0.75	1.0
			Males		
Brain					
axonal degeneration	0/4	0/4	1/4 (1)	4/4 (1.75)	4/4 (1.33)
neuronal focal degeneration	0/4	0/4	0/4	2/4 (1)	1/4 (1)
Spinal Cord					
axonal degeneration	0/4	0/4	0/4	4/4 (1.25)	4/4 (1)
Nerve					
axonal degeneration	0/4	0/4	3/4 (1.33)	4/4 (1.25)	4/4 (1.5)
Retina					
cellular degeneration	0/4	0/4	0/4	2/4 (1)	3/4 (1)
Optic nerve					
axonal degeneration	0/4	0/4	0/4	3/4 (1.33)	4/4 (1.25)
Muscle fiber					
focal degeneration	0/4	0/4	0/4	1/4 (1)	0/4
			Females		
Brain					
axonal degeneration	0/4	0/4	2/4 (1)	3/4 (1.33)	4/4 (1.75)
neuronal focal degeneration	0/4	0/4	0/4	0/4	2/4 (1)
Spinal Cord					
axonal degeneration	0/4	0/4	2/4 (1)	2/4 (1)	4/4 (1.5)
Nerve					
axonal degeneration	0/4	0/4	1/4 (1)	4/4 (1.25)	4/4 (1.5)
Retina					
cellular degeneration	0/4	0/4	0/4	1/4 (1)	3/4 (1)
Optic nerve					
axonal degeneration	0/4	0/4	0/4	1/4 (1)	3/4 (1.33)
Muscle fiber					
focal degeneration	0/4	0/4	1/4 (1)	3/4 (1)	0/4

[a] Data extracted from Study #90-612-0 and Study #90-612-01, Table B-6

[b] Numbers in parentheses indicate average severity (1 = very slight, 2 = slight or small, and 3 = moderate); averages calculated by reviewers

at 1.0 mg/kg/day. The incidence and severity of these lesions increased with dose. It is noteworthy that even though some of the animals showed clinical signs of neurotoxicity, none of the animals showed moderate or severe degenerative changes. The increases in severity were from very slight to slight.

At 0.75 mg/kg/day and above, optic tissues were also affected in both males and females. The lesions observed consisted of cellular degeneration in the retina and axonal degeneration in the optic nerve. As with the other nervous system tissues, the incidence and severity of these lesions increased with dose. The increases in severity were from very slight to slight.

Very slight muscle fiber degeneration was observed in females at doses as low as 0.5 mg/kg/day and in males at 0.75 mg/kg/day, but not in the animals at 1.0 mg/kg/day. The absence of this finding at 1.0 mg/kg/day was suggested to be due to the very early sacrifice of the dogs in this group, indicating that this effect developed as a consequence of relatively long-term neuronal degeneration.

B. DISCUSSION

The data presented in this study show that the nervous system was the principal target organ for MK-0244 toxicity in beagle dogs. The results of this study are consistent with effects seen in rats with deoxy avermectin. In the dog study, clinical signs of neurotoxicity including whole body tremors and stiffness of the hind legs were observed in females at doses as low as 0.5 mg/kg/day and in males at doses as low as 0.75 mg/kg/day. Additional signs of neurotoxicity observed in dogs at 0.75 mg/kg/day and above included mydriasis and difficulty in getting up. One female at 0.75 mg/kg/day also exhibited ataxia and hyperreactivity to touch, and at 1.0 mg/kg/day, both male and female dogs showed decreased motor activity. Histopathological examination of nervous system tissues showed axonal degeneration in the pons and medulla and in peripheral nerves (sciatic, sural, and tibial) at 0.5 mg/kg/day and above in both males and females. Axonal degeneration in the spinal cord was also observed in females at 0.5 mg/kg/day and above and in males at 0.75 mg/kg/day and above. Focal neuronal degeneration in the brain was also observed in males at 0.75 mg/kg/day and in both sexes at 1.0 mg/kg/day. Optical involvement was observed as cellular degeneration in the retina and axonal degeneration in the optic nerve at 0.75 mg/kg/day and above. Muscle fiber degeneration was also observed in females at 0.5 mg/kg/day and in both males and females at 0.75 mg/kg/day and appeared to be secondary to neuronal degeneration.

The neurotoxicity observed in dogs at 1.0 mg/kg/day, coupled with decreased food consumption and weight loss, resulted in the sacrifice of all dogs at this dose after 3 weeks of exposure. A new group of dogs was started on the study approximately 1 week after the sacrifice of the high-dose dogs. The dogs in this group received 0.75 mg/kg/day. However, the males at this dose were also sacrificed after only 7 weeks of exposure because of weight loss, decreased food consumption, and neurotoxicity. These unscheduled deaths indicate that the doses selected for this study

were too high. However, although the Subdivision F Guidelines recommend that no deaths occur in chronic toxicity studies in nonrodent species, the unscheduled sacrifices in this study do not compromise interpretation of the results of this study since a NOEL and LOEL could be determined. Similarly, although the Subdivision F Guidelines recommend that complete histopathological analyses be conducted on all dogs, the failure to examine tissues other than target organs in males and females at 0.25 mg/kg/day and in females at 0.5 mg/kg/day also is not believed to have affected the interpretation of this study because the data do not suggest that tissues other than nerve and muscle were affected.

Review of the final report and supporting data indicates that the conduct was adequate and the reporting of the results was accurate. Thus, despite the early sacrifices at the two highest doses, the study satisfies the intent of the guidelines and is classified as Core Minimum.

The NOEL for systemic toxicity is 0.25 mg/kg/day. The LOEL is 0.5 mg/kg/day based on axonal degeneration in brain and peripheral nerves and the resulting muscle fiber degeneration.