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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Mammalian Neurotoxicity - rat (82-7)

SHAUGHNESSY NO./TOX. CHEM. NO.: P.C. Code 122806

ACCESSION NO./MRID NO.: 427436-28

DP BARCODE/SUBMISSION NO.: D194567

TEST MATERIAL: MK-0243 Technical

SYNONYMS: Emamectin

STUDY NUMBER(S): TT #91-006-0; Lab Project ID: 618-244-TOX25

REPORT NUMBER: TT #91-006-0

SPONSOR: Merck & Co.

TESTING FACILITY: Merck Research Laboratories

TITLE OF REPORT: MK-0244. Fourteen-Week Dietary Neurotoxicity Study in Rats. TT #91-006-0

AUTHOR(S): Ronald L. Gerson

REPORT ISSUED: December 18, 1992

CONCLUSION: Randomized groups of 10/sex/dose of young adult Sprague-Dawley rats were fed dietary levels of emamectin to achieve dosages of 0, 0.25, 1.0, and 5.0 mg/kg/day for 14 weeks. Parameters measured included clinical signs, mortality, body weight food consumption, neurotoxicologic evaluations consisting of "Functional Observational Battery and Motor Activity" conducted at pretest and weeks 5, 9, and 13. At the termination of the study, all rats were anesthetized, exsanguinated, and perfused with 3% glutaraldehyde-3% paraformaldehyde fixative. Tissue sampling was limited to the central and peripheral nervous system, optic nerve, and skeletal muscle from all animals.

The NOEL for the study is 1.0 mg/kg/day (mid-dose). The LEL is 5.0 mg/kg/day (HDT).

However, dietary exposures averaged 85% of nominal doses and a more correct NOEL could be considered to be 0.85 mg/kg/day. There were no mortalities during the study. There were no clinical signs noted in the mid-dose or low-dose groups. Beginning in week 7, 2 high-dose males displayed excessive salivation, and one of these (91-0830) had slight whole body tremors. In following weeks, 7 of the remaining 9 high-dose rats developed tremors. Most of these rats displayed excessive salivation and rough, soiled coats. Two high-dose female rats developed tremors in week 11 that persisted for the remainder of the study.

Body weight gain was 512, 481, 485, and 382 grams for the control, low, mid, and high-dose groups, respectively. The 25.3% decrease in weight gain at the high-dose is considered treatment-related. In females, body weight gains were comparable between control and treated groups. Body weight gain was 233, 232, 245, 228, and 233 grams for control I, control II, low, mid, and high-dose groups, respectively.

Food consumption was most noticeably decreased in individual high-dose rats which displayed clinical signs. In week 9, food consumption was decreased in high-dose males by 9.1% in comparison to controls and in week 12, food consumption was decreased by 25.2% in high-dose males in comparison to controls. Food consumption was comparable between female control and treated rats of all dose groups and male control and low and mid-dose rats.

At the LEL of 5.0 mg/kg/day for neurotoxicologic measurements, the effects were more pronounced in males than females, and included neurotoxic endpoints such as mild tremors, posture, rearing, excessive salivation, fur appearance, gait, grip strength, mobility, and righting reflex. Motor activity was unaffected by treatment.

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At the high-dose, there was neuronal vacuolation in the brain and spinal cord and degeneration of nerve fibers in the spinal cord and sciatic nerve of both sexes of rats. Male rats were more affected than female rats. Skeletal muscle atrophy was also seen in some high-dose male rats. The NOEL for neuropathology is also 1.0 mg/kg/day.

Classification: **MINIMUM**

Testing Guideline Satisfied: 82-7

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: MK-0243 (MK-0244) {4" -epi methylamino avermectin derivative} **EMAMECTIN**

Description: None
Batch #(s), Other #(s): L-656,748-052-S002
Purity: 95.9%
Source: Merck & Co.
Vehicle (if applicable): Untreated rodent meal
Positive Control(s) (if applicable): None

2. Test Animals

Species and Strain (sexes): Crl:CD™(SD)BR Albino Rats
Age: 51 days (males), 53 days (females)
Source(s): Charles River Laboratories, Raleigh, NC

3. Procedure:

a. Dietary Preparation (if applicable): Appropriate amounts of test compound were weighed, mixed with small amounts of ground meal, Q.S. to approximately 1.1-1.4 kg of Purina Certified Rodent Chow and blended for 20 minutes. Portions of this premix were blended with the appropriate amount of rodent chow for 10 minutes to prepare each dosage level.

Frequency of preparation: Weekly and used within 10 days

Storage conditions: Room Temperature

Stability Analyses: Analyzed after 10 days at room temperature

Homogeneity Analyses: Samples of top, middle, and bottom portions of each batch of treated diet were assayed for MK-0244 by HPLC

Concentration Analyses: Samples of each batch of treated diet were analyzed for concentration of MK-0244 at low, mid, and high-dose levels

b. Basis For Selection of Dose Levels: Doses were based on NOEL of 5.0 mg/kg in Acute Oral Neurotoxicity Study in Rats (TT #89-0129-0)

c. Animal Assignment and Dose Levels: Rats were individually caged and had free access to Purina Certified Rodent Chow (meal) and drinking water. Food was withdrawn overnight prior to scheduled necropsy. Rats were randomized and assigned to the following groups, as shown below.

Test Group	Dose Administered	Main Study 14 weeks male	female
Control	1	10	10
Control	2*	0	10
1		10	10
2		10	10
3		10	10

*Additional female controls were used to obtain additional historical control FOB data in this sex.

d. Clinical Signs of Toxicity and Mortality: All rats were observed daily for mortality and clinical signs of toxicity.

e. Body Weight Determinations: Rats were weighed pretest and once weekly for the remainder of the study.

f. Food and/or Water Consumption: Food consumption was measured weekly generally on an approximate 6 day intake.

g. Functional Observational Battery and Motor Activity: Functional Observational Battery and Motor Activity testing were conducted on all animals pretest and on Days 29 or 30 (week 5), 57 or 58 (week 9), and 91 or 92 (week 13/14). The functional observational battery (FOB) consisted of a home cage and open field evaluation, grip strength, rear limb landing foot splay, rectal temperature, body weight, and tail flick measurements. Approximately 60 minutes after the start of the FOB testing, rats were placed in motor activity chambers and their horizontal activity, as determined by the total number of beam interruptions, were monitored automatically for a total of 60 minutes.

Measurement of spontaneous activity and/or CNS excitability included: home cage posture, convulsions, vocalization, removal difficulty, handling difficulty, fur appearance, arousal, and rearing.

Measurement of autonomic findings included: lacrimation, salivation, piloerection, defecation, urination, and pupil response.

Measurement of muscle tone equilibrium included: gait, gait score, mobility score, forelimb grip strength, hindlimb grip strength, landing (hindlimb) foot splay, and righting reflex.

Measurement of motor and sensory affective findings included: handling palpebral closure, approach response, click response, touch response, and tail flick.

Measurement of motor activity included horizontal activity.

FOB and motor activity data were statistically analyzed with significance indicated by $p \leq 0.05$.

i. Neuropathology: At the end of the 14 week study, all rats in the Control Group 1 and all rats treated with MK-0243 were anesthetized, exsanguinated, and perfused with 3% glutaraldehyde - 3% paraformaldehyde fixative. Tissue sampling was limited to the CNS and peripheral nerve, optic nerve and skeletal muscle from all animals.

The microscopic examination was limited to H & E or toluidine blue sections of the following tissues (* indicates plastic embedded tissues):

Brain:
6 transverse sections
Gasserian ganglion with trigeminal nerve*

Spinal Cord:
2 cross-sections (cervical and lumbar) and 1 longitudinal (cervical)
3 cervical and 2 lumbar nerve roots with dorsal root ganglion*

Sciatic Nerve Trunk:
Sciatic nerve branches-
Peroneal, tibial, and sural branches-cross and longitudinal sections*

Optic Nerve:
Longitudinal section
Skeletal Muscle:
Cross section - muscle groups of the hindlimb

All of the above tissues were examined from 6 rats/sex in the Control Group 1 and 5 mg/kg/day groups and 10 rats/sex in the 1 mg/kg/day group. Only paraffin embedded tissues from 6 rats/sex in the 0.25 mg/kg/day group were examined microscopically. Additionally, all gross lesions were examined embedded in paraffin, prepared and stained with H & E and examined microscopically.

j. Statistical Analyses: Functional Observational Battery and Motor Activity data were statistically analyzed with significance indicated by $p \leq 0.05$.

B. RESULTS:

1. Dietary Preparation: Dietary analyses measured homogeneity at bottom, middle, and top concentrations from 0-42 ppm. At week 1: For females, the results were 87.1% (top), 82.0% (middle) and 79.6% (bottom) at the high-dose (42 ppm); 77.1, 77.1, and 79.5% for the mid-dose (8.1 ppm); and 76.2, 76.2, and 76.2% for the low-dose (2.1 ppm). For males, the top, middle, and bottom levels were 92.6, 88.5, and 88.5% at the high-dose (41.7 ppm), 90.4, 88.0, and 88.0% at the mid-dose (8.3 ppm), and 95.2, 104.8, and 95.2% for the low-dose (2.1 ppm). For weeks 2-9 and 10-13, concentration analyses for both sexes, showed averages of 85.8% with a coefficient of variation of 8.4%. Analysis of rat feed samples (3 to 90 ppm) stored at room temperature for 3-4 days showed MK-0244 concentrations in close agreement (81.5 to 86.4%) with nominal concentrations. MK-0244 was found to be stable in rodent diet for 10 days.

2. Clinical Observations and Mortality: There were no mortalities during the study. There were no clinical signs noted in the mid-dose or low-dose groups. Beginning in week 7, 2 high-dose males displayed excessive salivation, and one of these (91-0830) had slight whole body tremors. In following weeks, 7 of the remaining 9 high-dose rats developed tremors. Most of these rats displayed excessive salivation and rough, soiled coats. Two high-dose female rats developed tremors in week 11 that persisted for the remainder of the study. The NOEL for clinical signs is 1.0 mg/kg/day (mid-dose).

3. Body Weight Determinations: Body weight gain was 512, 481, 485, and 382 grams for the control, low, mid, and high-dose groups, respectively. The 25.3% decrease in weight gain at the high-dose is considered treatment-related. In females, body weight gains were comparable between control and treated groups. Body weight gain was 233, 232, 245, 228, and 233 grams for control I, control II, low, mid, and high-dose groups, respectively. The NOEL for body weight gain is 1.0 mg/kg/day (mid-dose).

4. Food and/or Water Consumption: Food consumption was most noticeably decreased in individual high-dose rats which displayed clinical signs. In week 9, food consumption was decreased in high-dose males by 9.1% in comparison to controls and in week 12, food consumption was decreased by 25.2% in high-dose males in comparison to controls. Food consumption was comparable between female control and treated rats of all dose groups and male control and low and mid-dose rats. The NOEL for food consumption is 1.0 mg/kg/day (mid-dose).

5. Functional Observational Battery and Motor Activity:

The NOEL for neurotoxicologic measurements is 1.0 mg/kg/day (mid-dose). At the LEL of 5.0 mg/kg/day, the effects were more pronounced in males than females, and included neurotoxic endpoints such as mild tremors, posture, rearing, excessive salivation, fur appearance, ataxia, grip strength, mobility, and impaired righting reflex. Motor activity was unaffected by treatment.

Summary of FOB and Motor Activity

MALES

	Low Dose			Mid Dose			High Dose		
Week	**5**	**9**	**13**	**5**	**9**	**13**	**5**	**9**	**13**
No. Examined*	10	10	10	10	10	10	10	10	10
Study Parameters Are Listed Below with Number of Rats with Adverse Effects									
Spontaneous Activity, CNS Excitability-Trend								+	+
Home cage posture									2
Tremors								2	5
Vocalization									
Removal difficulty									
Handling difficulty									
Fur appearance								5	4
Arousal									
Rearing									6
Autonomic-Trend								+	+
Lacrimation									
Salivation								4	2
Piloerection									
Defecation									
Urination									
Pupil Response									
Muscle Tone Equilibrium-Trend								+	+
Gait									5
Gait score									5
Mobility Score									4
Forelimb grip strength								e	e
Hindlimb grip strength								e	e
Landing (Hindlimb) foot splay									
Righting reflex								1	5
Motor and Sensory Affective-Trend									
Handling palpebral closure									
Approach response									
Click response									
Touch response									

Tail flick

Motor Activity

Horizontal activity

e indicates an adverse effect on parameter
+ indicates a statistically significant trend

FEMALES

	Low Dose			Mid Dose			High Dose		
Week	**5**	**9**	**13**	**5**	**9**	**13**	**5**	**9**	**13**
No.Examined*	10	10	10	10	10	10	10	10	10
Study Parameters Are Listed Below with Number of Rats with Adverse Effect									
Spontaneous Activity, CNS Excitability-Trend									
Home cage posture									
Tremors									1
Vocalization									
Removal difficulty									
Handling difficulty									
Fur appearance									
Arousal									
Rearing									
Autonomic-Trend									
Lacrimation									
Salivation									
Piloerection									
Defecation									
Urination									
Pupil Response									
Muscle Tone Equilibrium-Trend								+	+
Gait									
Gait score									
Mobility Score									
Forelimb grip strength								e	e
Hindlimb grip strength									
Landing (Hindlimb) foot splay									
Righting reflex									1

Motor and Sensory Affective-Trend

Handling palpebral closure
Approach response
Click response
Touch response
Tail flick

Motor Activity

Horizontal activity

e indicates an adverse effect on parameter
+ indicates a statistically significant trend

* For some parameters, the number of rats evaluated was less than 10 since one or more rats could not be evaluated due to physical inability or uncooperative behavior.

6. Neuropathology: At the high-dose, there was neuronal vacuolation in the brain and spinal cord and degeneration of nerve fibers in the spinal cord and sciatic nerve of both sexes of rats. Male rats were more affected than female rats. Skeletal muscle atrophy was also seen in some high-dose male rats. The NOEL for neuropathology is 1.0 mg/kg/day. The table below demonstrates the incidence of the various lesions.

SUMMARY OF HISTOPATHOLOGY

DOSE (mg/kg/day)

	CONTROL		0.25		1.0		5.0	
	M	F	M	F	M	F	M	F
No.Necropsied	10	10	10	10	10	10	10	10
BRAIN								
No. Examined	6	6	6	6	10	10	6	6
neuron, cyto-plasmic vacuo-lation	0	0	0	0	0	0	6	6
SPINAL CORD								
No. Examined	6	6	6	6	10	10	6	6
white matter degeneration	1	1	0	0	1	0	6	4
neuron, cyto-plasmic vacuo-lation	0	0	0	0	0	0	6	6
SCIATIC NERVE								
No. Examined	6	6	6	6	10	10	6	6
degeneration	1	0	0	0	1	0	6	1
SKELETAL MUSCLE								
No. Examined	6	6	6	6	10	10	7	8
atrophy	0	0	0	0	0	0	3	0
chronic focal myositis	0	0	1	0	0	0	0	1

The grades of the neuronal lesions were very slight to moderate and the degree of atrophy of the skeletal muscle in the high-dose males were graded very slight, moderate and severe in the three affected animals.

7. Quality Assurance Measures: A Certification of Good Laboratory Practice was signed by the Study Director, Dr. Ronald J. Gerson, and dated December 18, 1992. A Quality Assurance Statement with Inspections and Report Audit Dates was signed by several members of the Quality Assurance Program.

C. DISCUSSION: The 14 week rat feeding neurotoxicity study was well conducted and the results indicate that emamectin is clinically and histologically neurotoxic at 5.0 mg/kg/day. The NOEL for all examined parameters is 1.0 mg/kg/day (mid-dose).