011308

FINAL

DATA EVALUATION REPORT

MK-0244 (Deoxy Avermectin)

Study Type: Chronic Oral Toxicity in Rats

Prepared for:

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# DATA EVALUATION REPORT

STUDY TYPE: Chronic Oral Feeding - Rat (83-1)

TOX. CHEM. NO.: New Chemical

P.C. CODE: 122806

MRID NO.: 428689-02

TEST MATERIAL: MK-0244 (deoxy avermectin)

SYNONYMS: 4'-epi methylamino avermectin derivative; L-656,748

STUDY NUMBER: TT#91-046-0;
618-244-TOX-48 (laboratory project identification number)

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

TESTING FACILITY: Merck Research Laboratories
Department of Safety Assessment
West Point, Pennsylvania

TITLE OF REPORT: MK-0244: Fifty-Three-Week Dietary Toxicity Study in Rats.
TT#91-046-0

AUTHOR: Ronald J. Gerson, Ph.D.

REPORT ISSUED: December 18, 1992

EXECUTIVE SUMMARY: In a chronic oral toxicity study, MK-0244 (deoxy avermectin; 95.9% pure) was administered in the diet at doses of 0, 0.1, 1.0, or 2.5 mg/kg/day in males and 0, 0.1, 1.0, or 2.5/5.0 mg/kg/day in females (the amount received by the high-dose females was lowered from 5.0 to 2.5 mg/kg/day at week 18 due to excessive treatment-related toxicity). The time-weighted average intake for the high-dose females was 3.3 mg/kg/day. Actual dietary levels were adjusted weekly using food consumption and body weight data from the previous week to maintain constant intake levels over the course of the study. In addition to the required parameters, a functional observational battery and motor activity were examined to assess compound-related neurotoxicity.

At 2.5 mg/kg/day, males exhibited an increased incidence of neuronal degeneration in the brain and spinal cord, decreased rearing, and an increased incidence of animals with low arousal. At 3.3 mg/kg/day, females had tremors, weight loss, an increased incidence of neuronal degeneration in the brain and spinal cord, and decreased grip strength. **A LOEL of 2.5 mg/kg/day is based on the evidence of neurotoxicity in males. The NOEL is 1.0 mg/kg/day.**

The study is core minimum and satisfies the guideline requirement for a 83-1a, chronic study in the rat. However, the study is core supplementary for a 83-1 chronic neurotoxicity study because of a failure to provide positive or historical control data to validate the methodology used to assess neurotoxicity; neuropathological analyses were not conducted according to the guideline recommendations for tissue preservation and several regions were not examined; and motor activity data were presented in such a way that habituation could not be assessed. No requirement exists for the submission of a chronic neurotoxicity study; a subchronic neurotoxicity study on MK-0244 has already been provided (MRID 427436 28; Core-minimum).

Special Review Criteria: (40 CFR 154.7) None

A. MATERIALS

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1. Test Material: MK-0244

   Description: Not reported

   Lot/Batch #: L-656,748-052S002

   Purity: 95.9% by weight (97.2-97.8%, area percent; of this, 92.5% represents the $B_{1a}$ form and 5.3% represents the $B_{1b}$ form. The $B_{1b}$ form differs from the $B_{1a}$ component by a methylene unit at the 26-carbon position; the ethyl group is a methyl group in the $B_{1b}$ form.)

   Stability of compound: The test material was stable in the diet for at least 35 days at room temperature over the concentration range of 0.1 to 100 ppm (see below).

   CAS number: Not reported

2. Vehicle and/or positive control: None

3. Test animals

   Species: Rat

   Strain: Crl:CD®(SD)BR

   Age and weight at study initiation: Males, 44 days, 188-234 g; females, 46 days, 154-201 g

   Source: Charles River Laboratories, Raleigh, NC

Housing: Individually caged in stainless steel wire cages

Environmental conditions:
Temperature: Not reported
Humidity: Not reported
Air changes: Not reported
Photoperiod: 12-hour light/dark

Acclimation period: Not reported

B. STUDY DESIGN

1. Animal assignment

Animals were assigned using a balanced random allocation scheme to the test groups in Table 1. The mean body weights of the various treatment groups at all doses were comparable at the start of the study.

TABLE 1. STUDY DESIGN

| Test Group | Dose in diet (mg/kg/day) | Male | Female |
|---|---|---|---|
| Control | 0 | 20 | 20 |
| Low (LDT) | 0.1 | 20 | 20 |
| Mid (MDT) | 1.0 | 20 | 20 |
| High (HDT) | 2.5 | 20 | |
| | 2.5/5.0[a] | | 20 |

[a]The high-dose females received 5.0 mg/kg/day for the first 17 weeks of the study; thereafter, the dose was lowered to 2.5 mg/kg/day due to excessive weight loss and tremors.

In addition to examination of the recommended parameters for a chronic oral toxicity study, neurotoxicologic evaluations (functional observational battery and motor activity) were conducted as described under methods and results.

Rationale for Dose Selection:

No rationale was provided for the selection of doses used in this study. However, a 14-week study in rats (Study number TT #88-059-0; MRID 427942-01) using deoxy avermectin showed no effects at 2.5 mg/kg/day and increased evidence of neurotoxicity (tremors, hindlimb splaying, urogenital staining, central and peripheral neuronal degeneration), and secondary muscle and bone atrophy at a time-weighted-average dose of 6.7 mg/kg/day in males and 7.1 mg/kg/day in females.

2. Diet preparation and analysis

Diets were prepared weekly by mixing appropriate amounts of MK-0244 with rat feed. A factor of 1.15 was used for dosage calculations. 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. Measurement of the homogeneity, stability, and actual concentration of the test material in the diets was conducted at the sponsor's analytical chemistry laboratory using HPLC with fluorescence detection after extraction using acetonitrile/triethylamine and derivatization using trifluoroacetic anhydride.

Results

Homogeneity Analysis: The coefficient of variation at each dietary level was ≤5.3%.

Stability Analysis: Analyses were conducted using diets prepared at 0.1 and 100 ppm (dietary levels for this study were within these limits). After 35 days at room temperature, no loss of test material was seen at 0.1 ppm and the loss of test material at 100 ppm was less than 15%.

Average Concentration Analysis: 89%-93% of nominal. Occasional measurements were more than 15% less than the target concentration but were not considered to affect the integrity of the study.

The actual concentration of the test material in the diets offered to the rats was measured nine times over the course of the experiment. The average nominal percentages at each test level were as follows:

TABLE 2. ACHIEVED DIETARY CONCENTRATIONS

| Expected Dose (mg/kg/day) | Percent Nominal (%) |
|---|---|
| **Males** | |
| 0.1 | 91.7 ± 6.45 |
| 1.0 | 89.0 ± 6.71 |
| 2.5 | 90.1 ± 4.17 |
| **Females** | |
| 0.1 | 92.3 ± 7.99 |
| 1.0 | 89.3 ± 6.65 |
| 2.5/5.0 | 93.3 ± 5.07 |

Data extracted from study TT#91-046-0, Appendix I

3. Animals received food (Purina Certified Rodent Chow) and water *ad libitum* throughout the acclimation and study periods.

4. Statistics - Statistical analyses were conducted in this study only on the data obtained in the neurotoxicology battery, terminal body weight, and organ weight data ($p \leq 0.05$; 1-sided). Neurotoxicology data were analyzed using trend analyses (Tukey et al. 1985). Trend analyses were conducted both for individual measures and for data grouped into four functional categories (autonomic, muscle tone and equilibrium, sensorimotor responses, central nervous system). The autonomic category included defecation, urination, pupil response, salivation, piloerection, and lacrimation. The muscle tone and equilibrium category included gait, gait score, mobility score, grip strength (fore and hind), footsplay, and righting reflex. The sensorimotor response category included approach response, click response, touch response, palpebral closure (with handling), and tail flick. The central nervous system category included rearing, arousal, posture, ease of removal, ease of handling, vocalizations, and fur appearance. Clonic and tonic convulsions, palpebral closure in the home cage, body weight, and body temperature were examined but not included in any category.

   Terminal body weight and organ weight data were analyzed for normality using the Wilk and Shapiro W statistic and for homogeneity using Levene's test. Where necessary, rankit transformations were conducted and data were analyzed for trend using Tukey's test.

5. A signed and dated quality assurance statement was present. A signed and dated GLP statement was present.

C. METHODS AND RESULTS

1. Observations

   All animals were inspected daily for signs of toxicity and mortality.

   Results

   a. Mortality/moribundity

      No treatment related deaths were observed. Between 1 and 4 deaths or unscheduled sacrifices occurred at each dose (Table 3); however, the number of deaths was not dose-related and no consistent cause of death was identified.

TABLE 3. MORTALITY INCIDENCE[a]

| Dietary Level (mg/kg/day) | Male | Female |
|---|---|---|
| 0 | 1 | 0 |
| 0.1 | 2 | 1 |
| 1.0 | 3 | 1 |
| 2.5 | 1 | NA |
| 2.5/5.0 | NA | 1 |

[a]Data from study report p. 35

b. Clinical observations

Females receiving 5 mg/kg/day showed increases in the incidence of tremors, unsteady gait, and unkempt coat (Table 4). Tremors were observed as early as study week 9 and were characterized as fine, whole-body tremors, which were often not visible except when handling animals. The tremors were usually present throughout the day. Up to 9 females (45%) exhibited tremors before lowering the dose. Unsteady gait was observed only in females that also had tremors, but unkempt coat and urine staining were observed both in females with and without tremors. The tremors, unsteady gait, and unkempt coat generally abated within 2-6 weeks after the dose-level was reduced to 2.5 mg/kg/day (at week 18). The urine staining was observed during weeks 7-11, and resolved prior to the reduction of dose levels. No treatment-related clinical signs were observed in low- or mid-dose females or in males at any dose level.

2. Body weight

Animals were weighed weekly throughout the study.

**Results** - No statistically significant differences were observed between the treated groups and controls with respect to body weight or body weight gain (calculated by the reviewers using analysis of variance; data not shown). However, a number of high-dose females lost weight between weeks 9 and 18 (Table 5). Many of the most severely affected females also had tremors. The decrease in body weight in these females occurred prior to or in conjunction with the onset of tremors. The weight loss and tremors were the bases for decreasing the dose from 5.0 to 2.5 mg/kg/day in high-dose females at week 18. After the reduction of the highest dose in females, the females recovered and overall body weight gains at 52 weeks were slightly higher than those of controls. Mean body weights and body weight gains among males at all dose levels were slightly greater than controls throughout the study (data not shown).

TABLE 4. Incidence of Clinical Observations in Rats Ingesting MK-0244 in the Diet for up to 1 Year[a]

| Parameter | Incidence by Dietary Level (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 1.0 | 2.5 |
| | | Males | | |
| Urine staining | 1/20 | 0/20 | 2/20 | 0/20 |
| Unkempt coat | 0/20 | 0/20 | 0/20 | 0/20 |
| Unsteady gait | 0/20 | 0/20 | 0/20 | 0/20 |
| Tremors | 0/20 | 0/20 | 0/20 | 0/20 |
| | | Females | | |
| Urine staining | 0/20 | 0/20 | 0/20 | 3/20 |
| Unkempt coat | 1/20 | 0/20 | 0/20 | 11/20** |
| Unsteady gait | 0/20 | 0/20 | 0/20 | 3/20 |
| Tremors | 0/20 | 0/20 | 0/20 | 9/20** |

[a] Data extracted from Study No. TT#91-046-0, Table A-139.
[b] Incidence refers to the number of animals exhibiting the effect, without regard to frequency of the observation

* Significantly different from control values, $p \leq 0.05$ using Fisher's exact test performed by the reviewers.
** Significantly different from control values, $p \leq 0.01$ using Fisher's exact test performed by the reviewers.

TABLE 5. Incidence of Mean Weight Loss or Gain Between Weeks 9 and 18 in Rats Ingesting MK-0244 in the Diet for Up to 1 Year[a]

| Parameter | Incidence by Dietary Level (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 1.0 | 2.5 |
| | | Males | | |
| Weight loss:167-84 g | 0/20 | 0/20 | 0/20 | 0/20 |
| Weight loss:0-83 g | 0/20 | 0/20 | 0/20 | 0/20 |
| Weight gain:1-83 g | 6/20 | 4/20 | 4/20 | 6/20 |
| Weight gain:84-167 g | 14/20 | 16/20 | 16/20 | 14/20 |
| | | Females | | |
| Weight loss:41-81 g | 0/20 | 0/19 | 0/20 | 2/20 |
| Weight loss:0-40 g | 0/20 | 0/19 | 0/20 | 6/20 |
| Weight gain:1-40 g | 16/20 | 15/19 | 11/20 | 10/20 |
| Weight gain:41-80 g | 4/20 | 4/19 | 9/20 | 2/20 |

[a]Data extracted from Study No. TT#91-046-0, Tables A-15 and A-16.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/day. Test article intake (mg MK-0244/kg body weight/day) was calculated weekly. These values were calculated using the weekly body weight and food consumption values and the nominal weekly dietary concentrations of MK-0244.

Results

a. Food consumption - No treatment-related effects on food consumption were observed. The decrease in mean body weight observed in females before dose reduction was not correlated with decreased food consumption during that time (weeks 9-18).

b. Compound consumption - The time-weighted average doses received by each group are as follows:

TABLE 6. TIME-WEIGHTED AVERAGE COMPOUND CONSUMPTION

| Expected Dose (mg/kg/day) | Actual Dose (mg/kg/day) |
|---|---|
| Males | |
| 0.1 | 0.1 ± 0.003 |
| 1.0 | 1.0 ± 0.029 |
| 2.5 | 2.5 ± 0.10 |
| Females | |
| 0.1 | 0.1 ± 0.005 |
| 1.0 | 1.0 ± 0.045 |
| 3.3[a] | 3.3 ± 1.2 |

Data from study TT91-046-0, Tables A5 and A6
[a]Time-weighted-average dose (17 weeks at 5 mg/kg/day and 35 weeks at 2.5 mg/kg/day)



4. Functional Observational Battery (FOB)

A functional observational battery (methods described in text of the study) was conducted using 10 rats/sex/dose pretest and during study weeks 14, 24, 38, and 51. The same 10 rats were examined at each interval. No replacements were made for animals dying during the study. No positive or historical control data were presented.

The parameters marked with an "X" below were examined.

Home Cage Observations

X Ease of removal from cage*
X Ease of handling/body tone*
X Palpebral closure*
Color of tears/deposits around eyes*
Respiration*
X Salivation*
X Appearance of fur*
X Convulsions/tremors*
X Piloerection*
X Vocalizations
X Excessive vocalization*
Exophthalmus*
X Posture/gait*
X Lacrimation*

Performance Measures

X Tail flick latency*
X Landing foot splay*
X Forelimb grip strength*
X Hindlimb grip strength*
X Rectal body temperature*

Open Field Observations

Posture*
X Gait*
X Arousal*
Circling*
X Stereotypy*
X Convulsions*
X Tremors*
X Urination*
X Defecation*
X Mobility
X Number of rears
X Bizarre behavior
X Vocalizations

Response Observations

X Righting reflex*
X Approach response*
X Pupil response*
X Touch response*

*Recommended by Subdivision F (March 1991) Guidelines

**Results** - The results of the functional observational battery (Tables 7a and 7b) were generally consistent with the daily clinical observations. At the 14-week examination, mild tremors and/or slightly soiled fur were observed in several high-dose females. In addition, a 30% decrease in forelimb grip strength was observed in high-dose females. The decrease in forelimb grip strength was not statistically significant, but was considered biologically significant because the most severely affected females were those that also had tremors (according to daily clinical observations made during week 14).

Note: A few discrepancies were noted in the reporting of tremors for two high-dose females. Tremors were reported for female #91-3341 in the daily clinical observations at the time of the functional observational battery at week 14 (study table A-139) and in the list of physical signs observed during the functional observational battery at weeks 14, 24, and 38 (study table A-140); however, the tables in the functional observational battery in which tremors were reported (study tables A-213 and A-233) did not indicate tremors in this female at any interval. Also, the functional observational battery (study tables A-213 and A-233) did not report tremors in female #91-3343 at week 14 while the daily clinical observations

TABLE 7a. Functional Observational Battery Data for Male Rats Ingesting MK-0244 in the Diet for up to 1 Year[a]

| | Functional Observational Battery Data by Dietary Level (mg/kg/day) | | | |
|---|---|---|---|---|
| Parameter/Interval | 0 | 0.1 | 1.0 | 2.5 |
| Clonic involuntary motor movement (Tremors)—home cage[b] | | | | |
| pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| week 14 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 24 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 38 | 0/10 | 0/9 | 0/10 | 0/10 |
| week 51 | 0/10 | 0/8 | 0/10 | 0/10 |
| Fur appearance (soiled)[c] | | | | |
| pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| week 14 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 24 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 38 | 0/10 | 1/9 (1.0) | 0/10 | 1/10 (1.0) |
| week 51 | 0/10 | 1/8 (1.0) | 2/10 (1.0) | 2/10 (1.0) |
| Forelimb grip strength (g)[d] | | | | |
| pretest | 795 ± 132 | 680 ± 84 (86%) | 761 ± 128 (96%) | 714 ± 106 (90%) |
| week 14 | 2357 ± 386 | 2130 ± 232 (90%) | 2062 ± 192 (87%) | 2144 ± 281 (91%) |
| week 24 | 2280 ± 352 | 1993 ± 289 (87%) | 1975 ± 264 (87%) | 2083 ± 287 (91%) |
| week 38 | 1983 ± 356 | 1768 ± 239 (89%) | 1912 ± 422 (96%) | 1855 ± 376 (94%) |
| week 51 | 2012 ± 403 | 1671 ± 389 (83%) | 1912 ± 463 (95%) | 1938 ± 244 (96%) |
| Arousal (decreased)[e] | | | | |
| pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| week 14 | 1/10 (1.0) | 0/10 | 1/10 (1.0) | 3/10 (1.0) |
| week 24 | 2/10 (2.0) | 4/10 (1.3) | 4/10 (1.3) | 5/10 (1.2) |
| week 38 | 3/10 (1.3) | 3/9 (1.7) | 4/10 (1.0) | 6/10 (1.0) |
| week 51 | 2/10 (1.5) | 4/8 (1.0) | 5/10 (1.2) | 9/10* (1.4) |
| Rearing (no./3 min.)[d] | | | | |
| pretest | 22 ± 1 | 21 ± 6 (95%) | 19 ± 9 (86%) | 21 ± 7 (95%) |
| week 14 | 14 ± 6 | 15 ± 3 (107%) | 14 ± 5 (100%) | 12 ± 7 (86%) |
| week 24 | 11 ± 6 | 7 ± 4 (64%) | 9 ± 6 (82%) | 7 ± 7 (64%) |
| week 38 | 8 ± 6 | 8 ± 6 (100%) | 7 ± 5 (88%) | 7 ± 6 (88%) |
| week 51 | 5 ± 4 | 6 ± 5 (120%) | 5 ± 4 (100%) | 2 ± 3* (40%) |

[a]Data extracted from Study No. TT#91-046-0, Appendix A, Tables 144, 162, 176, 180, and 200.
[b]Numbers in parentheses indicate average severity of tremors (0=absent, 1=chewing, 2=mild tremor, 3=severe tremor).
[c]Numbers in parentheses indicate average soiling of fur (0=normal, 1=slightly soiled, 2=very soiled).
[d]Numbers in parentheses indicate the percent of control.
[e]Numbers in parentheses indicate average arousal (1=slightly low, 2=low, 3=very low).

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

TABLE 7b. Functional Observational Battery Data for Female Rats Ingesting MK-0244 in the Diet for up to 1 Year[a]

| | Functional Observational Battery Data by Dietary Level (mg/kg/day) | | | |
|---|---|---|---|---|
| Parameter/Interval | 0 | 0.1 | 1.0 | 5.0/2.5[b] |
| Clonic involuntary motor movement (Tremors)–home cage[c] | | | | |
| pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| week 14 | 0/10 | 0/10 | 0/10 | 1/10* (2.0) |
| week 24 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 38 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 51 | 0/10 | 0/10 | 0/9 | 0/10 |
| Fur appearance (soiled)[d] | | | | |
| pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| week 14 | 0/10 | 0/10 | 0/10 | 4/10 (1.0) |
| week 24 | 0/10 | 0/10 | 0/10 | 1/10 (1.0) |
| week 38 | 0/10 | 0/10 | 0/10 | 1/10 (1.0) |
| week 51 | 0/10 | 0/10 | 0/9 | 0/10 |
| Forelimb grip strength (g)[e] | | | | |
| pretest | 758 ± 118 | 774 ± 141 (102%) | 738 ± 115 (97%) | 761 ± 89 (100%) |
| week 14 | 1508 ± 262 | 1580 ± 219 (105%) | 1558 ± 319 (103%) | 1049 ± 353 (70%) |
| week 24 | 1563 ± 310 | 1672 ± 307 (107%) | 1545 ± 295 (99%) | 1417 ± 376 (91%) |
| week 38 | 1527 ± 290 | 1617 ± 265 (106%) | 1453 ± 223 (95%) | 1532 ± 254 (100%) |
| week 51 | 1557 ± 209 | 1613 ± 375 (104%) | 1474 ± 336 (95%) | 1487 ± 257 (96%) |
| Arousal (decreased)[e] | | | | |
| pretest | 0/10 | 0/10 | 0/10 | 0/10 |
| week 14 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 24 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 38 | 0/10 | 0/10 | 0/10 | 0/10 |
| week 51 | 0/10 | 0/10 | 1/9 (1.0) | 0/10 |
| Rearing (no./3 min.)[f] | | | | |
| pretest | 24 ± 9 | 23 ± 8 (96%) | 26 ± 6 (108%) | 24 ± 8 (100%) |
| week 14 | 21 ± 5 | 25 ± 4 (119%) | 23 ± 5 (110%) | 23 ± 5 (110%) |
| week 24 | 21 ± 6 | 21 ± 8 (100%) | 21 ± 7 (100%) | 21 ± 6 (100%) |
| week 38 | 20 ± 5 | 20 ± 6 (100%) | 18 ± 6 (90%) | 19 ± 7 (95%) |
| week 51 | 16 ± 6 | 19 ± 4 (119%) | 17 ± 7 (106%) | 17 ± 7 (106%) |

[a]Data extracted from Study No. TT#91-046-0, Appendix A, Tables 143, 161, 175, 179, and 199.
[b]The diet concentration for high dose females was lowered in drug week 18 due to neurotoxic effects.
[c]Numbers in parentheses indicate average severity of tremors (0=absent, 1=chewing, 2=mild tremor, 3=severe tremor).
[d]Numbers in parentheses indicate average soiling of fur (0=normal, 1=slightly soiled, 2=very soiled).
[e]Numbers in parentheses indicate average arousal (1=slightly low, 2=low, 3=very low).
[f]Numbers in parentheses indicate percent of control.

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

(study table A-139) reported tremors in this female consistently between weeks 11 and 21. The reason for these discrepancies is unclear.

By week 24 (after the dose reduction in high-dose females), tremors were no longer observed, only one female had slightly soiled fur, and grip strength in affected females was returning toward control levels. Males did not show similar signs of toxicity. However, at the week-51 examination, high-dose males had significantly lower arousal and significantly decreased rearing. These effects were reflected in a significantly higher central nervous system index score in the high-dose males.

Other statistically significant effects were not considered to be treatment-related either because the values were within the normal physiological range or because interpretation was confounded by pretest differences between groups.

5. Motor Activity

Motor activity was measured in 10 rats/sex/dose pretest and during study weeks 14, 24, 38, and 51. The same 10 rats were examined at each interval. No replacements were made for animals dying during the study. No positive control data were presented. Motor activity was measured over a period of 60 minutes using a Digiscan Animal Activity Monitor (Model RXYCM(8), Omnitech Electronic Inc., Columbus, Ohio). Horizontal activity data were presented in the study report in 30-minute intervals.

**Results** - No treatment-related effects were observed on motor activity when compared to concurrent control or pretest activity values. Slight decreases were observed in total motor activity in mid- and high-dose males (Appendix 1), but these were not considered to be treatment related because of high variability of data (observed both pretest and at the intervals tested during treatment).

6. Ophthalmoscopic examinations

Eye examinations were conducted using indirect ophthalmoscopy after dilation of the pupils with a mydriatic agent. All animals were examined pretest, while only the control and high-dose rats were examined during study week 50.

**Results** - No treatment-related effects were observed.

7. Clinical Pathology

Hematology, blood chemistry, and urinalyses were performed on 10 rats/sex/dose during study weeks 13, 26, and 52. Animals were fasted and anesthetized with ether before collection of samples from the orbital sinus. The parameters marked with an "X" below were examined.

a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Blood clotting measurements
(Thromboplastin time)
(Clotting time)
(Prothrombin time)
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpusc. HGB conc. (MCHC)
X Mean Corpusc. volume (MCV)
Reticulocyte count

* Required for subchronic and chronic studies

Results - No treatment-related effects were observed.

b. Clinical Chemistry

Electrolytes
X Calcium*
X Chloride*
Magnesium
X Phosphate*
X Potassium*
X Sodium*

Enzymes
X Alkaline phosphatase (ALP)
Cholinesterase
X Creatinine phosphokinase
Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)*
X Serum aspartate aminotransferase (also SGOT)*
Gamma glutamyl transferase (GGT)
Glutamate dehydrogenase

Other
X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
Globulins
X Total protein*
X Glucose*
X Total bilirubin
X Triglycerides
X Cholesterol*
Serum protein electrophores

* Required for subchronic and chronic studies

Results - No treatment-related effects were observed.

8. Urinalysis

Urine was collected overnight in metabolism cages during study weeks 13, 26, and 52. The CHECKED (X) parameters were examined.

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

Results - No treatment-related effects were observed.

9. Sacrifice and Pathology

All rats received a complete gross examination at the time of the scheduled sacrifice. Tissues that are marked with an "X" below were examined histologically in the controls and high-dose animals. In addition, the brain, spinal cord, lungs, liver, kidneys, and all gross lesions were examined histopathologically in the low- and mid-dose rats. Brain sections were taken at the cerebral cortex, subcortical white matter, cerebellum, pons, and medulla. Spinal cord sections were taken at the cervical, thoracic, and lumbar levels. All tissues were preserved by immersion in 10% neutral buffered formaldehyde except the testes, which were fixed in Bouin's solution. All tissue sections were stained with hematoxylin and eosin. No special staining for nervous system tissue was used. Organs that are marked with an "XX" were also weighed at necropsy.

| Digestive System | Cardiovascular/Hematologic | Neurologic |
|---|---|---|
| Tongue | X Aorta* | XX Brain*+ |
| X Salivary glands* | X Heart* | X Peripheral nerve* (sciatic nerve) |
| X Esophagus* | X Bone marrow* | X Spinal cord* (three levels) |
| X Stomach* | X Lymph nodes* | X Pituitary* |
| X Duodenum* | X Spleen* | X Eyes* |
| X Jejunum* | X Thymus* | Glandular |
| X Ileum* | Urogenital | XX Adrenal gland* |
| X Cecum* | XX Kidneys*+ | Lacrimal gland |
| X Colon* | X Urinary bladder* | X Mammary gland* |
| X Rectum* | XX Testes*+ | X Parathyroids*++ |
| XX Liver*+ | X Epididymides | X Thyroids*++ |
| Gall bladder* | X Prostate | Other |
| X Pancreas* | X Seminal vesicles | X Bone* |
| Respiratory | X Ovaries*+ | X Skeletal muscle* |
| X Trachea* | X Uterus* | X Skin |
| X Lung* | | X All gross lesions and masses* |
| Nose | | |
| Pharynx | | |
| Larynx | | |

* Required for subchronic and chronic studies.
\+ Organ weight required for subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

Note: *In situ* perfusion of animals and paraffin and/or plastic embedding of nervous system tissue were not performed as recommended in the Subdivision F, March 1991 guidelines for a Neurotoxicity Screening Battery. Therefore, the sensitivity of the histopathological analyses to detect neuronal lesions was not optimal. Also, a number of neurological tissues (e.g., dorsal root ganglia and additional peripheral nerves such as the sural or tibial) were not examined.

**Results -**

a. Organ weight - No treatment-related effects were observed.

b. Gross pathology - No treatment-related lesions were observed.

c. Microscopic pathology - Histopathologic analysis showed very slight or slight neuronal degeneration in the brain and spinal cord of high-dose males and females (Table 8). Detailed pathology reports for each individual animal were not provided, but the report stated that the brain lesions appeared primarily in the olivary nucleus and reticular formation in the pons and medulla. The spinal cord lesions were reported to have been located in the dorsal and ventral horns. Lesions appeared as increased vacuolation of the cytoplasm and swelling the neuronal cell body. Chromatolysis and peripheral displacement of the nucleus was also observed.

D. DISCUSSION

Review of the final report and supporting data indicates that the nervous system is the primary target organ for MK-0244 in male and female rats. Both functional and histopathological effects were observed. At a dose of 5 mg/kg/day, female rats exhibited tremors, weight loss, and decreased grip strength. Lowering the high dose in females to 2.5 mg/kg/day allowed recovery from the weight loss and overt signs of neurotoxicity. However, at the terminal pathological examination, females at the 5.0/2.5 mg/kg/day dose had very slight neuronal degeneration in the brain and spinal cord. Males exhibited no overt signs of neurotoxicity until the end of the study when those at 2.5 mg/kg/day had decreased rearing and a higher incidence of animals with low arousal. The males at 2.5 mg/kg/day also had very slight or slight neuronal degeneration in the brain and spinal cord. Similar effects were not observed in males or females at 1.0 mg/kg/day.

Reasonably good concordance was obtained between the daily clinical observations and findings made in the functional observational battery; however, some discrepancies were noted. For example, two high-dose females that had tremors at week 14 according to the daily clinical observations were not observed to have tremors during the functional observational battery conducted at week 14. Also, decreased arousal in high-dose males was not noted during the daily clinical observations, but was noted in most high-dose males tested during the functional observational battery performed at week 51.

The results of this study are generally consistent with those observed in a 14-week oral toxicity study in rats (MRID number 427942-01), although not all effects observed in the 14-week study were observed in this 1-year study. In the 14-week study tremors; hindlimb splaying; urogenital staining; neuronal degeneration in the spinal cord, optic nerve, and sciatic nerve; neuronal vacuolation in the brain and spinal cord; skeletal muscle atrophy; and decreased body weight and food consumption were observed in males and females at time-weighted-average doses of 6.7 and 7.1 mg/kg/day, respectively. The NOEL in the 14-week study was 2.5 mg/kg/day. The current study did not show skeletal muscle atrophy, optic or sciatic nerve degeneration, or decreased food consumption. Furthermore, hindlimb splaying could not confidently be attributed to exposure to MK-0244 in the current study. The differences in these results are probably the result of the lower doses used in the current

TABLE 8. Incidence of Neuronal Degeneration in Rats Ingesting MK-0244 in the Diet for up to 1 Year[a,b]

| Parameter | Incidence by Dietary Level (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 1.0 | 2.5 |
| **Males** | | | | |
| Brain<br>Neuronal degeneration | 0/20 | 0/20 | 0/20 | 9/20** (1.1) |
| Spinal cord<br>Neuronal degeneration | 0/20 | 0/20 | 0/20 | 4/20 (1.3) |
| **Females** | | | | |
| Brain<br>Neuronal degeneration | 0/20 | 0/20 | 0/20 | 19/20** (1.0) |
| Spinal cord<br>Neuronal degeneration | 0/20 | 0/20 | 0/20 | 2/20 (1.0) |

[a]Data extracted from Study No. TT#91-046-0, Table B-13.
[b]Numbers in parentheses indicate the average severity score (1=very slight, 2=slight or small, 3=moderate, 4=marked, 5=severe).

** Significantly different from control values, $p \leq 0.01$ using Fisher's exact test performed by the reviewers.

study. The results of the 1-year study are more consistent with that of a subchronic neurotoxicity study (MRID 427436-28) in which tremors, weight loss, and neuropathy were observed with a NOEL of 1.0 mg/kg/day and a LOEL of 5.0 mg/kg/day (doses tested were 0, 0.25, 1.0, and 5.0 mg/kg/day).

E. STUDY DEFICIENCIES

The functional observational battery and motor activity analyses conducted at four intervals during the study were intended to supplement the assessment of neurotoxicological effects. However, no positive control or historical control data were provided to fully assess the sensitivity of the testing methodology. (The study authors noted that validation of the testing procedures could be found in studies TT #90-056-0 [MRID 427436-28] and TT #90-088-0. However, neither study was provided for review.) Also, motor activity data were presented in 30-minute intervals, precluding an effective analysis of habituation.

The sensitivity of the histopathological analyses for neurotoxicological effects was limited because of the absence of positive control data or optimal tissue preservation and staining techniques (as recommended in the March 1991 guidelines). Thus, it is possible that subtle microscopic neurotoxicological changes may have occurred at doses lower than those at which effects were observed in this study.

Additional study deficiencies include failure to obtain organ weights of the ovaries and failure to report environmental (housing) conditions

This study satisfies the guideline requirements for a chronic oral toxicity study in rats and is classified as Core Minimum for chronic oral toxicity because of minor study deficiencies (failure to report ovary weights and environmental conditions). For the deficiencies reported above, this study does not satisfy the guideline requirements for a chronic oral neurotoxicology screen in rats and is classified as Core Supplementary for chronic oral neurotoxicity. However, the study did not specifically state that it was being submitted to fulfill the requirements for a chronic neurotoxicity study nor is a chronic neurotoxicity study currently required (the subchronic neurotoxicity requirement has been satisfied).

APPENDIX 1. Total Motor Activity Data for Rats Ingesting MK-0244 in the Diet for up to 1 Year[a]

| Interval | Total Motor Activity Data by Dietary Level (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 1.0 | 2.5 |
| | | **Males** | | |
| Pretest | 5818 | 4396 (76)[b] | 5142 (88) | 5998 (103) |
| Week 14 | 5775 | 5499 (95) | 4962 (86) | 4977 (86) |
| Week 24 | 4093 | 4454 (109) | 3503 (86) | 3944 (96) |
| Week 38 | 3160 | 3149 (100) | 3172 (100) | 2560 (81) |
| Week 51 | 3218 | 3389 (105) | 2748 (85) | 2635 (82) |
| | | **Females** | | |
| Pretest | 6682 | 7065 (106) | 6374 (90) | 5858 (83) |
| Week 14 | 6170 | 8014 (130) | 6250 (101) | 7707 (125) |
| Week 24 | 6560 | 8586 (131) | 6359 (97) | 7580 (116) |
| Week 38 | 4336 | 6537 (151) | 5205 (120) | 5696 (131) |
| Week 51 | 4081 | 5209 (128) | 4985 (122) | 4345 (106) |

[a] Data extracted from Study No. TT#91-046-0, Tables A-209 and A-210. Values represent total no of beam breaks per hr. session.
[b] Numbers in parentheses indicate percent of control.