FINAL

DATA EVALUATION REPORT

MK-0243 (Benzoate salT) 2/24/1994

Study Type: Developmental Toxicity (Rabbit)

Prepared for:

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1

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DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity (Rabbit); Guideline Series 83-3

EPA IDENTIFICATION NUMBERS
P.C. Code: 122806
Tox Chem. No.: New Chem

MRID Nos.: 427436-36, 427436-34 (Rangefinding), and 427436-35 (Rangefinding)

TEST MATERIAL: MK-0243 (Benzoate salt)

SYNONYM: Deoxy avermectin

SPONSOR: Merck Research Laboratories, Merck & Co., Inc., Three Bridges, NJ

STUDY NUMBER: 618-244-TOX33 (TT #89-715-0)

TESTING FACILITY: Merck Research Laboratories, Merck & Co., Inc., West Point, PA, and Three Bridges, NJ

TITLE OF REPORT: Oral Developmental Toxicity Study in Rabbits

AUTHOR: J.M. Manson

REPORT ISSUED: December 22, 1992

CONCLUSIONS: In a developmental toxicity study, New Zealand White rabbits were administered MK-0243 via gavage at daily doses of 0, 1.5, 3, or 6 mg/kg/day on gestation days (GDs) 6-18, inclusive.

Maternal NOEL = 3 mg/kg/day
Maternal LOEL = 6 mg/kg/day based on a significant trend towards decreased body weight gain during the dosing period and increased clinical signs (mydriasis and decreased pupillary reaction)

Developmental NOEL = 6 mg/kg/day
Developmental LOEL = not determined

CORE CLASSIFICATION: Minimum data. This study meets the minimum guideline requirements (83-3) for a developmental toxicity study in rabbits.

2

A. MATERIALS

Test Compound

Purity:	94.2%
Description:	Not reported
Lot number:	L-656,748-038W002 (benzoate salt)
Receipt date:	Not reported
Contaminants:	None reported
Storage:	Not reported

Vehicle: Deionized water

Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	Hazleton Research Products, Inc., Denver, PA
Age:	Approximately 25 weeks on GD 0
Weight:	3086-4124 g on GD 0
Males used:	Not reported

B. STUDY DESIGN

This study was designed to assess the potential of MK-0243 to cause developmental toxicity in rabbits when administered daily via gavage on GDs 6-18, inclusive.

Mating: Females were artificially inseminated with at least 0.25 mL of diluted semen from a pooled sample collected from at least three untreated males. Prior to insemination, females received 25 USP units of human chorionic gonadotropin (Organon, Inc.). The study author did not indicate if and how long animals were acclimated to laboratory conditions prior to insemination.

Animal husbandry: Animals received 125 g of food daily (Purina Certified Rabbit Chow® #5322) and tap water ad libitum throughout the study. Temperature was maintained at 17°-23°C. A 12-hour light/dark cycle was maintained. Percent humidity and number of air changes/hour were not reported.

Group arrangement: Animals were assigned to the following dose groups using a computer-generated randomization procedure based on body weight:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	18
Low-dose	1.5	18
Mid-dose	3	18
High-dose	6	18

3

Dose administered: Dosing solutions were prepared daily and were stirred constantly during dosing. Doses (adjusted for purity) were administered in a volume of 4 mL/kg; they were based on the most recently recorded body weight. Analyses for concentration and homogeneity of the test material in the vehicle were conducted once during the study. Stability analysis had been performed earlier.

Dose rationale: Doses were selected based on the results of two range-finding studies (Study Nos. 618-244-TOX31 and 618-244-TOX32). In the first study, six nonpregnant rabbits per group received the test material (as the HCl salt) by gavage for 13 days at daily doses of 1.37, 2.74, 5.48, or 10.96 mg/kg/day. All animals at 10.96 mg/kg/day were sacrificed moribund because of increased clinical signs and reduced body weight. Compound-related effects were not observed at any other dose level.

In the second study, 10 presumably pregnant rabbits per group received the test material (as the benzoate salt) by gavage at daily doses of 2, 4, 6, or 8 mg/kg/day on GDs 6-18, inclusive. Compound-related clinical signs were observed at 8 mg/kg/day including tremors, soft and/or small feces, and no urine. Maternal body weight gain (at 6 and 8 mg/kg/day during dosing) and food consumption (at 8 mg/kg/day on GDs 16, 19, and 22) were significantly ($p \leq 0.05$) below control. Developmental toxicity was not observed at any dose level.

Observations: Animals were observed twice daily during the dosing period and once daily during the remainder of the study for mortality, moribundity, and clinical signs. Body weight data were recorded on GDs 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, and 28. Food consumption data were recorded for the following intervals: GDs 0-1, 3-4, 6-7, 9-10, 12-13, 15-16, 18-19, 21-22, 24-25, and 27-28. On GD 28, animals were sacrificed by an intravenous injection of T-61 euthanasia solution (Hoechst-Roussel) and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- Gross pathology examination of abdominal and thoracic cavities
- Number of corpora lutea
- Number of implantation sites
- Numbers of resorptions (early and late) and live and dead fetuses

The study author did not indicate how early embryonic loss was detected.

Examination of live fetuses included the following:

- Individual fetal weight and sex
- External anomalies
- Visceral anomalies
- Free-hand coronal sectioning of the head for approximately one-half of the fetuses

- Skeletal anomalies

Statistical analysis: The following method was used.

- Body weight gain; food consumption; numbers of implantation sites, post implantation loss, and live fetuses; and fetal weight--Trend analyses for linear, quadratic, and/or time responses

Compliance: The following statements were provided.

- A signed Statement of No Data Confidentiality Claims, dated January 14, 1993

- A signed Statement of Compliance with EPA GLPs, dated December 22, 1992, and January 12 and 14, 1993

- A signed, but not dated, Quality Assurance Statement

C. RESULTS

Test Material Analysis

Analyses for concentration and homogeneity of the test material in the vehicle revealed values ranging from 99% to 110% of target. Results of a previously conducted analysis for stability were not submitted.

Maternal Toxicity

Mortality: No mortality was observed at any dose level.

Abortion: No compound-related abortions were reported at any dose level. One female in the control group aborted on GD 19.

Clinical observations: Compound-related clinical signs were observed at 6 mg/kg/day. A summary of clinical signs is presented in Table 1. Nine animals experienced mydriasis and 16 animals experienced decreased pupillary reaction at 6 mg/kg/day. Incidental signs, noted in all dose groups, included blood in pan, pulling fur, soft feces, scab, rales, and sore mouth.

Body weight: Compound-related effects on body weight gain were observed at 6 mg/kg/day. A summary of body weight gain data for selected intervals is presented in Table 2. Significant trends towards decreasing body weight gain were observed during GDs 12-19 (48% of control; data not shown) and 6-19 (60% of control; Table 2) at 6 mg/kg/day. For GDs 19-28 and 6-28, body weight gain decreased nonsignificantly to 82% and 72% of control, respectively. Body weights were similar at all times in all other dose groups.

Food consumption: No compound-related effects on food consumption (g/animal/day) were observed at any dose level. Sporadic, but significant trends towards decreased food consumption were observed at 6 mg/kg/day on GDs 10 and 22. Since these decreases were not consistent

5

TABLE 1. Clinical Signs in Rabbits Exposed to MK-0243 During Major Organogenesis[a]

Observation	Observation for Each Dose Level (mg/kg/day)			
	0	1.5	3	6
Number of animals	18	18	18	18
Blood in pan	0	1	0	0
Pulling fur	1	0	0	2
Soft feces	6	2	4	0
Ocular discharge	1	0	0	1
Scab	1	0	0	0
Increased urine	1	1	1	0
Rales	0	1	0	0
Mydriasis	0	0	0	9
Decreased pupillary reaction	0	0	0	16
Sore mouth	0	0	0	1

[a]Data were extracted from Study No. 618-244-TOX33, Table 4.

TABLE 2. Mean Body Weight Gain (g) of Rabbits Exposed to MK-0243 During Major Organogenesis[a,b]

Dose Group (mg/kg/day)	Pre-Dosing Period[c] (GDs 0-6)	Dosing Period (GDs 6-19)	Post-Dosing Period (GDs 19-28)	Gestation Period (GDs 6-28)
0	69	150	132	282
1.5	52	158	151	309
3	62	166	114	280
6	48	90*	112	202

[a]Data were extracted from Study No. 618-244-TOX33, Table 2.

[b]Standard deviations were not provided.

[c]Calculated by the reviewers; analyzed by ANOVA

*Significant trend ($p \leq 0.05$) through indicated dose

7

during the dosing period and were <5%, they were not considered to be biologically relevant.

Gross pathology observations: No compound-related gross findings were observed.

Cesarean section observations: No compound-related effects were observed at any dose level. A summary of cesarean section observations is presented in Table 3.

Developmental Toxicity

No compound-related effects were observed at any dose level. A summary of external, visceral, and skeletal malformations is presented in Table 4.

External examinations: No external malformations or variations were observed in any dose group.

Visceral examinations: Visceral malformations consisted of the following: one fetus in the control group (retrocaval ureter); three fetuses from different litters at 1.5 mg/kg/day (ventricular septal defect, retrocaval ureter, or an epididymal malformation); one fetus at 3 mg/kg/day (cerebral malformation); and two fetuses from different litters at 6 mg/kg/day (retrocaval ureter or hydrocephalus). Visceral variations, occurring with similar incidences in all dose groups, consisted of variations in lung lobation.

Skeletal examinations: Skeletal malformations consisted of the following: three fetuses from different litters in the control group (thoracic vertebra malformation, agenesis of the rib, or a pelvic bone malformation); two fetuses from different litters at 1.5 mg/kg/day (pelvic bone malformation); two fetuses from different litters at 3 mg/kg/day (thoracic vertebra or caudal vertebra malformation); and five fetuses from five litters at 6 mg/kg/day (lumbar vertebra, sternebra, or pelvic bone malformation). Skeletal variations, occurring with similar incidences in all dose groups, included vertebra and sternebral variations as well as incomplete ossification of various bones.

D. DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) which is included with the evaluation of the study. All but one criterion were satisfied. Criterion 6 (analytical chemistry of dosing solutions) was only partially fulfilled; results of a previously conducted analysis for stability were not reported.

Test Material Analyses

Analyses for concentration of the test material in the vehicle revealed values within ±10% of nominal. The test material was shown to be homogenous in the vehicle.

TABLE 3. Cesarean Section Observations in Rabbits Exposed to MK-0243 During Major Organogenesis[a]

Parameter	Observation for Each Dose Level (mg/kg/day) 0	1.5	3	6
No. animals inseminated	18	18	18	18
No. animals pregnant	15	17	15	17
Pregnancy rate (%)[b]	83	94	83	94
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	2	1	3	1
No. aborted	1	0	0	0
Does with live litters	15	17	15	17
Total corpora lutea[b]	136	169	135	164
Corpora lutea/doe[c]	9.1 ± 1.1[d]	9.9 ± 1.8	9.0 ± 1.5	9.6 ± 2.1
Total implantations	123	143	115	146
Implantations/doe[c]	8.2 ± 1.8	8.4 ± 2.2	7.7 ± 2.3	8.6 ± 2.2
Total live fetuses	120	139	110	143
Live fetuses/doe[c]	8.2 ± 1.8	8.2 ± 2.3	7.3 ± 2.2	8.4 ± 2.2
Total resorptions	3	4	4	1
Early resorptions	2	3	2	1
Late resorptions	1	1	2	0
Resorptions/doe[c]	0.2 ± 0.5	0.2 ± 0.4	0.3 ± 0.6	0.1 ± 0.2
Total dead fetuses	0	0	1	2
Dead fetuses/doe	0	0	0.1 ± 0.2	0.1 ± 0.3
Fetal weight/litter (g)[c]				
Males	35.1 ± 4.4	35.1 ± 5.0	38.1 ± 4.0	35.6 ± 4.8
Females	33.6 ± 4.4	34.6 ± 5.8	36.8 ± 5.3	34.0 ± 4.5
Preimplantation loss (%)	10	15	15	11
Postimplantation loss (%)[b]	2	3	4	2
Sex ratio (% male)[b]	41	50	54	51

[a]Data were extracted from Study No. 618-244-TOX33, Tables 5 and 13.

[b]Calculated by the reviewers; not analyzed

[c]Calculated by the reviewers; analyzed by ANOVA

[d]Mean ± S.D.

TABLE 4. Incidences of Malformations in Fetuses Exposed to MK-0243 During Major Organogenesis[a,b]

Findings[c]	Observation for Each Dose Level (mg/kg/day)			
	0	1.5	3	6
Number of fetuses examined	120 (15)	139 (17)	110 (15)	143 (17)
Total number of fetuses with any malformation	4 (3)	5 (5)	3 (3)	7 (7)
External Malformations				
Total number of fetuses with any external malformation	0	0	0	0
Visceral Malformations				
Ventricular septal defect	0	1	0	0
Retrocaval ureter	1	1	0	1
Epididymal malformation	0	1	0	0
Hydrocephalus	0	0	0	1
Cerebral malformation	0	0	1	0
Total number of fetuses with any visceral malformation	1	3 (3)	1	2 (2)
Skeletal Malformations				
Thoracic vertebra malformation	1	0	1	0
Lumbar vertebra malformation	0	0	0	2 (2)
Caudal vertebra malformation	0	0	1	0
Agenesis of rib	1	0	0	0
Sternebral malformation	0	0	0	2 (2)
Pelvic bone malformation	1	2 (2)	0	1
Total number of fetuses with any skeletal malformation	3 (2)	2 (2)	2 (2)	5 (5)

[a]Data were extracted from Study No. 618-244-TOX33, Tables 6, 7, 8, and 14.

[b]Numbers in parentheses indicate number of litters.

[c]More than one type of malformation may be found in one fetus.

Maternal Toxicity

Compound-related maternal toxicity was observed at 6 mg/kg/day. It was manifested as a significant trend towards decreased body weight gain during the dosing period and an increased incidence of clinical signs (mydriasis and decreased pupillary reaction). Based on these results, the NOEL and LOEL for maternal toxicity were 3 and 6 mg/kg/day, respectively.

Developmental Toxicity

Developmental toxicity (including resorptions and dead fetuses, fetal altered growth, and anomalies) was not observed in this study. Consequently, the NOEL for developmental toxicity was 6 mg/kg/day; the LOEL was not determined.

Study Design/Reporting Deficiencies

Study design deficiencies included the following:

- Gravid uterine weights were apparently not recorded, and therefore, corrected maternal body weight/body weight gain could not be determined.

- The study did not indicate how or if early embryonic loss was detected, and therefore, the pregnancy rates may be incorrect.

Reporting deficiencies included the following:

- A protocol (and deviations) was not submitted.

- Results from a previously conducted stability analysis of the test material in the vehicle were not reported.

- Standard deviations for maternal and fetal body weights and maternal food consumption data were not reported.

E. CORE CLASSIFICATION: Core Minimum Data.

Maternal NOEL = 3 mg/kg/day
Maternal LOEL = 6 mg/kg/day based on decreased body weight gain and increased clinical signs

Developmental Toxicity NOEL = 6 mg/kg/day
Developmental Toxicity LOEL = not determined

F. RISK ASSESSMENT: Not applicable

ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	Y/N	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an asterisk (*) are supplemental, may not be required for every study.