FINAL

2/24/1994

DATA EVALUATION REPORT

MK -0243 (Benzoate salt)

Study Type: Developmental Toxicity Study (Rat)

Prepared for:

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Clement Number: 436 and 437
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DATA EVALUATION REPORT

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

EPA IDENTIFICATION NUMBERS

Tox Chem. No.: New Chem.
PC. Code: 122806
MRID Nos.: 427436-32 and 427436-31 (Rangefinding)

TEST MATERIAL: MK-0243 (Benzoate salt)

SYNONYM: Deoxy avermectin

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

STUDY NUMBER: 618-244-TOX29 (TT #89-716-0)

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

TITLE OF REPORT: Oral Developmental Toxicity Study in Rats

AUTHOR: J.M. Manson

REPORT ISSUED: December 22, 1992

CONCLUSIONS: In a developmental toxicity study, Crl:CD (SD) BR rats were administered MK-0243 via gavage at daily doses of 0, 2, 4, or 8 mg/kg/day on gestational days (GDs) 6-18, inclusive.

Maternal NOEL = 2 mg/kg/day
Maternal LOEL = 4 mg/kg/day based on a significant trend towards decreased body weight gain during the dosing period

In addition to a significant trend towards decreased body weight gain at 8 mg/kg/day, incidences of the following clinical signs increased: unkempt coat, tremors, convulsion, and few or no feces.

Developmental NOEL = 4 mg/kg/day
Developmental LOEL = 8 mg/kg/day based on altered growth and an increased incidence of supernumerary rib.

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

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A. MATERIALS

Test Compound

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

Vehicle: Deionized water

Test Animals

Species: Rat
Strain: Sprague-Dawley Crl:CD (SD) BR
Source: Charles River Breeding Laboratories, Raleigh, NC
Age: Approximately 11.5 weeks on GD 0
Weight: 229-320 g on GD 0
Males used: Same strain as females

B. STUDY DESIGN

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

Mating: Females were mated in a 1:1 ratio with males of the same strain. The day a copulatory plug was observed was considered to be GD 0. The study author did not indicate if/how long animals were acclimated to laboratory conditions prior to mating.

Animal husbandry: Food (Purina Certified Rodent Chow® #5002) and tap water were available ad libitum throughout the study. A 12-hour light/dark cycle was maintained. The temperature was maintained at 20°-27°C. Percent humidity and number of air changes/hour were not reported.

Group arrangement: Animals were randomly 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	25
Low-dose	2	25
Mid-dose	4	25
High-dose	8	25

Dose administered: Doses were administered daily via gavage from GD 6 through 19 in a volume of 5 mL/kg. Individual doses were adjusted for purity and calculated based on the most recently recorded body weight. Analyses for concentration and homogeneity were performed during the first week of dosing. Concentration was again determined during the third week. Stability of the test material in the vehicle had been determined previously.

Dose rationale: Doses were selected based upon the results of a range-finding study (No. 618-24-TOX28). In this study, 10 mated female rats per group received the test material via gavage at daily doses of 1.25, 2.5, 5.0, or 10.0 mg/kg/day on GDs 6-17, inclusive. All animals at 10.0 mg/kg/day were sacrificed moribund because of loss of body weight and overt signs of toxicity consisting of tremors, unkempt coat, and nasal discharge. Neither maternal nor developmental toxicity was observed at ≤5 mg/kg/day.

Observations: Animals were observed three times daily during the dosing period and once daily on GDs 0 and 20 for mortality, moribundity, and clinical signs of toxicity. Body weight data were recorded on GDs 0, 6, 8, 10, 12, 14, 16, 18, and 20. Food consumption data were recorded for the following intervals: GDs 3-5, 6-8, 9-11, 12-14, 15-17, and 18-20. On GD 20, all animals were sacrificed by carbon dioxide asphyxiation and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross pathology observations of the 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 examination of all fetuses
- Visceral examination of approximately one-half of the fetuses in each litter and of all fetuses with external malformations
- Skeletal examination of all fetuses

Statistical analysis: The following methods were used.

- Body weight gain, number of implantation sites, number of live fetuses, fetal weight, and developmental anomalies--Trend analyses for linear, quadratic, and/or time responses

Compliance: The following statements were submitted.

- 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 Analyses

Concentration and homogeneity analyses of the test material in the vehicle, revealed values ranging from 100% to 113% and from 94% to 113%, respectively 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 abortions were reported at any dose level.

Clinical observations: Compound-related clinical signs were observed at 8 mg/kg/day. A summary of clinical signs is presented in Table 1. These signs consisted of unkempt coat (three dams), few or no feces (two dams), tremors (15 dams), and convulsion (two dams). Incidental signs, occurring in all dose groups, consisted of alopecia, mass, and ocular discharge.

Body weight: Compound-related effects on body weight gain were observed at 4 and 8 mg/kg/day. A summary of body weight gain data is presented in Table 2. Significant trends towards decreased body weight gain were observed on GDs 14-20 at 4 mg/kg/day (87% of control) and 8 mg/kg/day (65% of control) and on GDs 6-14 and 16-20 at 8 mg/kg/day (71% and 67% of control, respectively). Body weights were similar at all times in all dose groups.

Food consumption: Effects on food consumption (g/animal/day) were inconsistently observed at all dose levels. A summary of food consumption data is presented in Table 3. A significant trend towards increased food consumption was noted on GDs 7-8 at 4 and 8 mg/kg/day (115%) and on GDs 10-11 in all dose groups (107%-119%). On GDs 16-17 and 19-20, a significant trend towards decreased food consumption at 8 mg/kg/day (87% and 83% of control, respectively) was noted. These trends are probably treatment related, although their biological significance is minor.

Gross pathology observations: No compound-related gross findings were observed at any dose level.

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

Observation	Observation for Each Dose Level (mg/kg/day) 0	2	4	8
Number of animals	25	25	25	25
Alopecia	1	0	0	1
Ocular discharge	0	1	0	1
Mass	0	0	2	0
Unkempt coat	0	0	0	3
Few or no feces	0	0	0	2
Tremors	0	0	0	15
Convulsion	0	0	0	2

[a]Data were extracted from Study No. 618-244-TOX29, Table 4.

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

Dose Group (mg/kg/day)	Pre-Dosing Period[c] (GDs 0-6)	Dosing Period (GDs 6-14)	Dosing Period[d] (GDs 14-20)	Gestation Period[d] (GDs 6-20)
0	38	42	92	135
2	39	51	90	141
4	36	48	80*	128*
8	35	30*	60*	90*

[a]Data were extracted from Study No. 618-244-TOX29, Table 2.
[b]Standard deviations were not provided.
[c]Calculated by the reviewers; analyzed by ANOVA
[d]Animals were dosed until GD 19.

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

TABLE 3. Food Consumption (g/animal/day) in Rats Exposed to MK-0243 During Major Organogenesis[a,b]

Dose Group (mg/kg/day)	Pre-Dosing Period (GDs 3-5)	Dosing Period (GDs 6-8)	Dosing Period (GDs 9-11)	Dosing Period (GDs 15-17)	Post-Dosing Period (GDs 18-20)
0	26	26	27	30	29
2	25	28	29*	31	30
4	26	30*	31*	32	29
8	25	29*	32*	26*	24*

[a]Data were extracted from Study No. 618-244-TOX29, Table 3.
[b]Standard deviations were not provided.

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

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Cesarean section observations: Compound-related effects were observed at all dose levels. A summary of cesarean section observations is presented in Table 4. Fetal body weight decreased nonsignificantly at 8 mg/kg/day in both males (4%) and females (6%). When the reviewers recalculated and reanalyzed these body weights, the female weights decreased significantly below control at 2, 4, and 8 mg/kg/day. The number of resorptions per litter increased at 8 mg/kg/day. Since this increase was neither significant nor different from the historical control value, it was considered to be a normal biological variation.

Developmental Toxicity

Compound-related increased incidences of skeletal variations were observed at 8 mg/kg/day. A summary of fetal external, visceral, and skeletal anomalies is presented in Table 5.

External examinations: External malformations were observed in one fetus each per dose group. They consisted of malformed tails (control group and 4 mg/kg/day), micrognathia (2 mg/kg/day), and cleft palate (8 mg/kg/day. Variations consisted of hematomas in two fetuses from different litters in the control group.

Visceral examinations: Visceral malformations were observed in one fetus each at 2 and 4 mg/kg/day. They consisted of ventricular hypoplasia (2 mg/kg/day) and agenesis of the testis (4 mg/kg/day). Variations, occurring as single events in all dose groups, consisted of reduced ductus arteriosus, diffuse hemorrhagic kidney, and variations in liver lobation.

Skeletal examinations: Skeletal malformations in the control group consisted of two fetuses from two different litters with sacral vertebra malformations and seven fetuses in another litter with hypoplastic rib. Three of these seven fetuses also had malformed lumbar vertebrae. At 2 mg/kg/day, one fetus had a sacral vertebra malformation and a second fetus from a different litter had a hypoplastic rib. At 8 mg/kg/day, one fetus had thoracic and lumbar vertebrae malformations and a second fetus from a different litter had a sacral vertebra malformation.

Significant trends towards increased incidences of skeletal variations were noted at 8 mg/kg/day. These variations consisted of supernumerary ribs, incomplete ossification of the pelvic bone, and incomplete ossification of the skull bone. The incidence of wavy ribs also increased at 8 mg/kg/day; however, this increase was nonsignificant. Incidental skeletal variations, occurring in all dose groups, included vertebral count variation, cervical rib, and various incomplete ossification sites.

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 criteria except for one were satisfied. Criterion 6 (analytical

TABLE 4. Cesarean Section Observations in Rats Exposed to MK-0243 During Major Organogenesis[a]

Parameter	Observation for Each Dose Level (mg/kg/day) 0	2	4	8
No. animals mated	25	25	25	25
No. animals pregnant	21	19	21	21
Pregnancy rate (%)	84	76	84	84
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	4	6	4	4
No. aborted	0	0	0	0
Gravid uterine weight (g)	--[b]	--	--	--
Dams with live litters	21	19	20	21
Total corpora lutea[c]	378	319	346	392
Corpora lutea/dam[c]	18.0 ± 1.5[d]	17.7 ± 1.3	17.3 ± 1.9	18.7 ± 2.7
Total implantations	348	320	318	348
Implantations/dam[c]	16.6 ± 1.5	16.8 ± 2.1	15.1 ± 4.8	16.6 ± 1.9
Total live fetuses	344	315	312	332
Live fetuses/dam[c]	16.4 ± 1.5	16.6 ± 2.2	14.9 ± 5.1	15.8 ± 2.0
Total resorptions	4	4	6	16
Early resorptions	4	4	6	15
Late resorptions	0	0	0	1
Resorptions/dam[c]	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.5	0.8 ± 1.5
Total dead fetuses	0	1	0	0
Dead fetuses/dam[c]	0	0.1 ± 0.2	0	0
Fetal weight/litter (g)[e]				
Males	3.8 ± 0.6	3.7 ± 0.3	3.7 ± 0.3	3.6 ± 0.5
Females	3.7 ± 0.5	3.6 ± 0.3*	3.5 ± 0.3*	3.5 ± 0.4*
Preimplantation loss (%)	7	5	9	10
Postimplantation loss (%)[c]	1	2	2	5
Sex ratio (% male)[c]	50	53	54	45

[a]Data were extracted from Study No. 618-244-TOX29, Tables 5 and 13.

[b]Not reported

[c]Calculated by the reviewers; not statistically analyzed

[d]Mean ± S.D.

[e]Calculated by the reviewers; analyzed by ANOVA and Scheffe's test

*Significantly different from control ($p \leq 0.05$)

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TABLE 5. Incidences of Malformations and Skeletal Variations in Fetuses Exposed to MK-0243 During Major Organogenesis[a]

Findings[c]	Observation for Each Dose Level (mg/kg/day)[b] 0	2	4	8
External Malformations				
Number of fetuses examined	344 (21)	315 (19)	312 (20)	332 (21)
Micrognathia	0	1	0	0
Cleft palate	0	0	0	1
Tail malformation	1	0	1	0
Total number of fetuses with any external malformation	1	1	1	1
Visceral Malformations				
Number of fetuses examined	176 (21)	163 (19)	161 (20)	173 (21)
Ventricular hypoplasia	0	1	0	0
Agenesis of testis	0	0	1	0
Total number of fetuses with any visceral malformation	0	1	1	0
Skeletal Malformations				
Number of fetuses examined	344 (21)	315 (19)	312 (20)	332 (21)
Thoracic vertebra malformation	0	0	0	1
Lumbar vertebra malformation	3 (1)	0	0	1
Sacral vertebra malformation	2 (2)	1	0	1
Missing vertebra	1	0	0	0
Hypoplastic rib	7 (1)	1	0	0
Total number of fetuses with any skeletal malformation	9 (3)	2 (2)	0	2 (2)
Total number of fetuses with any malformation	9 (3)	4 (4)	2 (2)	3 (3)
Skeletal Variations				
Wavy rib	0	0	0	5 (3)
Supernumerary rib	36 (8)	36 (12)	41 (13)	73* (18)
Incomplete ossification of the pelvic bone	2 (2)	4 (2)	3 (2)	35* (7)
Incomplete ossification of the skull bone	0	1	3 (2)	4* (4)

[a]Data were extracted from Study No. 618-244-TOX29, Tables 6-9 and 14.

[b]Numbers in parenthesis indicate number of litters.

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

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

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 that, overall, values were within ± 13% of target. Homogeneity analysis showed that the test material was homogenous in the vehicle.

Maternal Toxicity

Compound-related maternal toxicity was observed at 4 and 8 mg/kg/day as evidenced by significant trends towards decreased body weight gain during the dosing period at 4 and 8 mg/kg/day and by an increase in clinical signs (tremors, convulsion, unkempt coat, and few or no feces) at 8 mg/kg/day. Based on these results, the maternal NOEL and LOEL were 2 and 4 mg/kg/day, respectively.

Developmental Toxicity

Compound-related developmental effects were observed at 8 mg/kg/day. They were manifested as a significant trend toward increased incidence of supernumerary rib and altered growth (slightly decreased fetal body weights in both sexes which may have caused decreased ossification in the pelvic and skull bones). Based on these results, the NOEL and LOEL for developmental toxicity were 4 and 8 mg/kg/day, respectively.

Study Design/Reporting Deficiencies

Study design deficiencies included the following:

- Gravid uterine weights were apparently not recorded, and therefore, corrected maternal body weight/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: Minimum Data.

Maternal NOEL = 2 mg/kg/day
Maternal LOEL = 4 mg/kg/day based on decreased body weight gain and increased clinical signs

Developmental Toxicity NOEL = 4 mg/kg/day
Developmental Toxicity LOEL = 8 mg/kg/day based on an increased incidence of supernumerary rib and altered growth

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.