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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 618-EUP-18, Avermectin B_1; Rat and Rabbit Teratology Studies. Additional Histopathology Data for Subchronic rat and dog studies
Caswell No. 63AB
Accession No. 249152

TO: George LaRocca
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THRU: Christine F. Chaisson, Ph.D. CFChaisson 12/5/84
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FROM: William Dykstra, Ph.D. William Dykstra 12/5/84
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Action Requested: Review rat and rabbit teratology studies to determine teratogenic potential. Review additional histopathological data on subchronic dog and subchronic rat studies to upgrade to core-minimum data. 12/6/84

Recommendations:

1. The rat teratology is acceptable as supplementary data. An explanation of increased maternal body weight in the treated group is required to be submitted. Historical control data for maternal effects and fetal effects are required to be submitted to complete the evaluation of the study.

2. The rabbit teratology study is acceptable as supplementary data. Historical control data for maternal effects and fetal effects are required to be submitted to complete the evaluation of the study.

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3. The additional histopathology data of tissues from rats and dogs at all dose-levels are acceptable. The NOEL for the subchronic rat study is 0.4 mg/kg/day and the NOEL for the subchronic dog study is 0.25 mg/kg/day. The studies are acceptable as core-minimum data. The data were previously requested in memo of June 16, 1982 from W. Cyrstea to G. LaRocca to support the previously established findings based on histopathology of the high-dose and control animals only. With respect to the rat study, the dosages were 0, .01, 0.2 and 0.4 mg/kg/day. Histological examination of control and high-dose animals were performed. The additional histological evaluation at the low and mid-dose demonstrated that no histopathological effects were present and the NOEL for the study is 0.4 mg/kg/day. In the subchronic dog study, the dosages were 0,0.25, 0.5, 2.0 and 8.0 mg/kg/day. Histopathology was conducted on high-dose and control animals. No histological effects were found. Histological examination of animals at 0.25, 0.5, and 2.0 mg/kg/day levels showed no compound-related effects.

Background:

Avermectin B_1, also known as MK-0936, is orally a Toxicity Category I pesticide. The LD_{50} varies from 1.5 mg/kg in mice to 10.6 mg/kg in rats. Pregnant mice, orally gavaged, have a NOEL of 0.05 mg/kg/day. At 0.075 mg/kg/day, one pregnant mouse died after four days.

Avermectin B_1 is teratogenic in mice producing cleft palate and ablepharia. The NOEL for terata in mice is 0.2 mg/kg/day.

Studies assessing reproduction, and chronic toxicity are currently in progress and may be available in May, 1985. Toxicology "one liners" are attached which present the current data base for Avermectin.

Review:

1. MK-0936. Oral Range Finding and Teratogenic Studies in Rats and Rabbits (Merck Institute for Therapeutic Research Studies, TT #82-705-1; TT #82-705-0; 11/10/82).

   a. Oral Range Finding Study (TT #82-705-1)

      Test material: Technical MK-0936; 94%; Lot #: L-676, 863-00V50.

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Groups of 10 mated Sprague-Dawley rats were orally gavaged with doses of 0, .25, 0.5, 1.0 and 2.0 mg/kg/day of test material in sesame oil during days 6-17 of gestation. Parameters evaluated were toxic signs, body weight, and pregnancy. Dams were sacrificed at day 20 of gestation.

Statistical analyses of the maternal body weight changes in the range-finding study were performed using an IBM 370 computer. Statistical significance at $P = 0.05$ was based on an analysis of variance or covariance using a least significant difference procedure after normalizing for non-parametric data. When necessary, a covariance analysis was used to adjust for the effect of Day 6 weights on maternal body weight changes.

Results: One rat at 2.0 mg/kg/day was sacrificed at day 18 of gestation and displayed weakness, loss of body weight and tremors prior to sacrifice. Body weight gains were increased in the 0.25 and 0.50 mg/kg/day groups during the study.

Conclusion: The results of this study were used to establish dose levels for the main study. Although only one rat died at 2.0 mg/kg, the rat displayed treatment related signs. This level is considered adequate to determine maternal toxicity in light of an LD_{50} of 10.6 mg/kg. Additionally, the NOEL is 0.05 mg/kg for maternal toxicity in pregnant mice. At the LEL of 0.075 mg/kg, one mouse died after four doses. Maternal NOEL is 1.0 mg/kg/day, maternal LEL is 2.0 mg/kg/day.

Classification: Supplementary data.

b. Teratogenic Evaluation in Rats (TT #82-705-0).

Test material: Technical MKO-0936; 94% purity; Lot # L-676, 863-00V50.

Groups of 25 impregnated Sprague-Dawley rats were orally gavaged with doses of 0, 0.4, 0.8, and 1.6 mg/kg/day in sesame oil during days 6-19 of gestation. Parameters evaluated for dams were toxic signs, body weight, and pregnancy status. On day 20, the dams were sacrificed, and corpora lutea, implantations, and resorptions were recorded. Fetuses were examined for external abnormalities, sex, and were weighed. Visceral examination was performed

on one-third of the fetuses in each litter. Free-hand sections of the head of one-third of the fetuses in each litter was performed. All fetuses were processed, stained with Alizarin red all examined for skeletal abnormalities. Stataistical analyses of and maternal body weight changes, live fetal weights, percent preimplantation loss, rate of resorptions, number of implants per pregnant female, and number of live fetuses per pregnant female in the teratogenic study were performed using an IBM 370 computer. Statistical significance at P = 0.05 was based on an analysis of variance or convariance using a least significant difference procedure after normalizing for non-parametric data. When necessary, a covariance analysis was used to adjust for the effect of Day 6 weights on maternal body weight changes and the effect of time of sacrifice on average live fetal weights.

Results: No deaths and toxic signs were recorded during the study. Body weight gain was noted for all test groups during the study. The number of pregnant dams available for evaluation were 23, 24, 24 and 24 for the control, low dose, mid-dose and high-dose groups. There were no effects on implantations and percent preimplantation loss.

Resorptions were increased in the mid-dose group only. The total number of fetuses, sex-ratio, number dead (zero for all groups) and fetal body weight were unaffected by treatment. External abnormalities were noted in 1, 0, 2, 2 fetuses for these same groups. One fetus at 0.8 mg/kg/day had multiple fetal malformations. These malformations are not considered treatment-related. Visceral examination did not reveal any treatment-related malformations or variations. Skeletal examination showed the following results as presented in the report:

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Results of Skeletal Fetal Examination

	Control	(mg/kg/day) 0.4	0.8	1.6
Fetuses				
No. Examined	319	320	279	326
No. with Malformations	2	7	2	1
No. of Malformations	2	7	3	1
No. with Variations	44	42	45	72
No. of Variations	45	44	46	77
Litters				
No. Examined	23	24	24	24
No. with Fetal Malformations	2	2	2	1
No. with Fetal Variations	13	18	14	16
Type and Number of Fetal Alterations [a,b]				
Sternebral Malformation	0	0	0	1
Agenesis of Rib (M)	0	1	0	0
Hypoplastic Rib (M)	1[1]	3	0	0
Wavy Rib (M)	1	0	1[2,1]	0
Missing Vertebra (M)	0	3	0	0
Scapula Malformation	0	0	1[2]	0
Cervical Rib (V)	0	2	0	0
Lumbar Rib (V)	44	41	45	72
Lumbar Count Variation	1	1	1	5

[a]M = Malformation; V = Variation

[b]Identical superscripts within the same column on external, visceral and skeletal alteration tables denote malformations from the same fetus.

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At the low-dose, 0.4 mg/kg/day, 7 fetuses (2 litters) had skeletal malformations; agenesis of rib (1 fetus), hypoplastic rib (3 fetuses) and missing vetebrace (3 fetuses). These finding were not considered compound related, since they were not dose-related. The distribution of the skeletal varation denoted as lumar rib was 44/319 (13.8%) 41/320, (12.8%) 45/279 (16.1%) and 72/326 (22.1%) for the control, low-, mid-, and high-dose groups, respectively.

These incidences in the high-dose group are considered compound-related.

No treatment-related differences in fetal incomplete ossification were noted.

Additional, the skeletal lumbar count variation for fetuses was 1, 1, 1, and 5 for the control, low-, mid-, and high-dose groups, respectively.

Conclusion: The teratogenic potential was negative. The maternal NOEL is the high-dose group (1.6 mg/kg/day). The fetotoxic LEL is the high-dose group. The fetotoxic NOEL is considered to be 0.8 mg/kg, the mid-dose group. Maternal toxicity was not demonstrated in this study (body weight gain is not considered toxicity), but maternal toxicity (death) at 2.0 mg/kg/day in the range-finding study and other data are considered as sufficient evidence for an acceptable classificaiton of this study.

Classification: Supplementary data. (a) Historical control data for maternal and fetal effects are required to be submitted for complete evaluation of the study.

2. MK-0936; Oral Range Finding Study in Pregnant Rabbits and Oral Teratogenic Study in Rabbits (Merck Institute for Therapeutic Reserach, (TT #82-707-1, TT #82-706-0).

   a. Oral Range Finding Study in pregnant rabbits (TT #82-706-1).

      Test material: MK-0936; 94% purity, Lot number L-767, 863-00V50.

      Groups of 10 impregnated New Zealand white rabbits were orally gavaged with doses of 0, 0.5, 1.0, 2.0 and 3.0 mg/kg/day of test material in sesame oil from days 6-18 of gestation. Toxic signs, body weight and pregnancy status were evaluated during the study. Dams were sacrificed on day 28 of gestation.

      Statistical analyses of the maternal body weight changes in the range-finding study were performed using an IBM 370 computer. Statistical significance at $p = 0.05$ was based on a analysis of variance or covariance using a least significant difference procedure

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after normalizing for nonparameteric data. When necessary, a covariance analysis was used to adjust for the effects of Day 6 weights on maternal body weight changes.

Results: One rabbit died in the 3.0 mg/kg/day group on day 16 of gestation.

Four rabbits aborted. One in the 1.0 mg/kg/day group on day 20 of gestation, one in the 2.0 mg/kg/day group on day 27 of gestation, and two in the 3.0 mg/kg/day group on days 26 and 27 of gestation.

Toxic signs and decreased body weight were observed in females from the 2.0 mg/kg/day group only. Increased body weight was noted in the 1.0 mg/kg/day group.

Conclusion: Maternal LEL is 3.0 mg/kg/day. Maternal NOEL is 2.0 mg/kg/day. The abortions noted in the 1.0 and 2.0 mg/kg/day groups in study are not considered treatment-related due to the small sample size.

Classification: Supplementary data.

b. Oral Teratogenic Study in Rabbits (TT #82-706-01).

Test material: MK-0936; 94% purity; Lot No. L-767, 863-0[illegible]50.

Groups of 18 impregnated New Zealand white rabbits were orally gavaged with doses of 0, 0.5, 1.0 and 2.0 mg/kg/day of test material on days 6-27 of gestation.

The rabbits were sacrificed on day 28 of gestation. The parameters evaluated were toxic signs, body weight and pregnancy status. Corpora lutea, implantations and resorptions were counted. All fetuses were given exteranl examination, sexed, weighed and examined for visceral and skeletal abnormalities.

Statistical analyses of maternal body weight changes, live fetal weights, percent preimplantation loss, rate of resorptions, number of implants per pregnant female, and number of live and dead fetuses per pregnant female in the teratogenic study were performed using an IBM 370 computer. Statistical significance at $P = 0.05$ was based on an analysis of variance or covariance using a least significant difference procedure after normalizing for nonparameteric data. When necessary, a covariance analyais was used to adjust for the effects a Day 6 weights on maternal body weight changes and the effects of litter size on live fetal weight.

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Results: The following number of live pregnant rabbits were available for evaluation in the study: 15 (control), 13 (low-dose), 11 (mid-dose), and 15 (high-dose).

One non-pregnant rabbit at 0.5 mg/kg/day died on day 26, one non-pregnant rabbit at 1.0 mg/kg/day died on day 27, and one pregnant rabbit at 2.0 mg/kg/day was sacrificed in poor condition on day 20 of gestation. Abortions occurred as one (control), two (low-dose), one (mid-dose), and one (high-dose) but are not considered treatment-related since there was no dose-response relationship.

The following number of rabbits were alive but not pregnant: 2 (control), 2 (low-dose), 5 (mid-dose), and 1 (high-dose).

Treatment-related decreases in body weight, food consumption and water consumption were noted in most rabbits of the 2.0 mg/kg/day group.

Implants, percent implantation loss and resorptions were unaffected by treatment. The number of fetuses available for evaluation were 97 (control), 90 (low-dose), 95 (mid-dose) and 121 (high-dose). Five dead fetuses at the mid-dose (1.0 mg/kg/day) group were significantly increased above control (0), but were not considered treatment-related since there was no dose-related relationship in this parameter. Sex-ratio and fetal body weight were not affected by treatment.

External examination showed a treatment-related increase at 2.0 mg/kg/day (maternally toxic level) in malformations in comparison to controls and other groups. Cleft palate (12 fetuses, 1 litter), omphalocele (2 fetuses, 1, litter), clubbed forefeet (5 fetuses, 3 litters) occurred in the high-dose group, whereas omphalocele (1 fetus, 1 litter) were present in the control. Additionally, clubbed forefoot was present in 2 fetuses (1 litter) from the 1.0 mg/kg/day group.

Visceral examination did not show any treatment-related effect. However, there was a slight increase in the number of fetuses with variation of lung lobation in the 0.5 (2), 1.0 (2) and 2.0 mg/kg/day groups (3) in comparison to controls (1).

With respect to skeletal malformations, the number of litters (fetuses) with skeletal malformations was 0(0) in controls, 3 (4) in the low-dose, 1(2) in the mid-dose and 3(5) in the high-dose.

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At the low dose, 2 fetuses had caudal vertebrae malformations, one fetus had fused ribs, and one fetus had both branched ribs and thoracic vertebrae malformation. At the mid-dose, one fetus had craniostenosis and another had vertebral malformation. These malformations at 2.0 mg/kg/day occurred in litters and in fetuses that displayed treatment-related external abnormalities also. However, it should be noted that 2.0 mg/kg/day is a maternally toxic dose level.

Fetotoxicity at 2.0 mg/kg/day was evident as incompletely ossified sternbrae, metacarpals, and forefoot phalanges.

Conclusion: The findings at 2.0 mg/kg/day of external and skeletal malformations occured at a level which produced maternal toxicity. Therefore the malformations at this level can be attributed to maternally toxic effects. The NOEL for maternal toxicity is 1.0 mg/kg/day.

However, the low dose of 0.5 mg/kg/day, four-fold less than the high-dose level, produced 3/13 litters with skeletal terata in comparison to 0/15 litters in the control and 3/15 litters in the high-dose.

The results at the low dose are suggestive of compound related effects.

However, the narrow dose range and the high toxicity of the chemical make a clear separation of maternal effects and fetal effects at lower doses very difficult to assess in a species as variable as rabbits. The NOEL for fetotoxicity is 1.0 mg/kg.

Classification: Supplementary data. (a) Historical control data for maternal and fetal effects are required to be submitted for complete evaluation of the study.

3. Supplemental Histology Reports: Requested in memo of 6/16/82.

a. C-076 (Bla): Fourteen-Week Oral Toxicity Study in Rats Following *In Utero* Exposure, TT #77-043-0.

Results: The additional histopathology provided did not show any treatment-related effects. NOEL is 0.4 mg/kg/day.

Classification: Core minimum data.

b. C-076 (Bla): 18-Week Oral Toxicity Study in Dogs; TT #76-073-0.

Results: The additional histopathology provided did not show any treatment-related effects. NOEL is 0.25 mg/kg/day.

Classification: Core minimum data.