CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MAR 15 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Avermectin B1 (Abamectin) - Additional Toxicology Studies with Delta-8,9-Isomer and Polar Degradates - PP#8F3592 and 6H5550 (Citrus); PP#7F3500 (Cotton)

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FROM: William Dykstra, Reviewer William Dykstra 3/10/89
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: George T. LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (H7505C)

THRU: Edwin R. Budd, Section Head
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Requested Action

Review toxicology studies submitted by Merck Sharp and Dohme with Delta-8,9-Isomer and Polar Degradates of Avermectin B1. These studies are in support of permanent tolerances on citrus and cotton. See attachment.

Conclusions and Recommendations

1. The microbial mutagenicity study with the delta-8,9-isomer is acceptable. The delta-8,9-isomer was not mutagenic in the Ames assay.

2. The rat teratology study with the delta-8,9-isomer is a Core-Minimum study. The NOEL for maternal and developmental toxicity is 1.0 mg/kg/day (HDT).

3. The single generation rat reproduction study with the delta-8,9-isomer is considered a Supplementary study. The NOEL for the delta-8,9-isomer one-generation rat reproduction study is 0.40 mg/kg/day (HDT) with respect to parental females and pups only (male rats were not treated). This study is acceptable in fulfillment of the requirement for a "shortened or abbreviated" reproduction study in rats to be performed on the delta-8,9-isomer. See Toxicology Branch memorandum by William Dykstra, dated September 2, 1987, regarding a meeting with Merck Sharp and Dohme on August 3, 1987 (attachment, page 2).

4. The microbial mutagenicity study with the polar degradates is acceptable. The polar degradates were not mutagenic in the Ames assay.

5. The teratology study with polar degradates in CF_1 mice is acceptable as a Core-Minimum study. The NOEL for maternal and developmental toxicity is 1.0 mg/kg/day (HDT).

Review

1. Delta-8,9-Isomer, L-652-280; Microbial Mutagenesis Assay (Ames Test); MSD No. TT#87-8046; June 7, 1988; by L. Gordon (MRID No. 407134-02).

   Test Material - L-652,280-000N005; Delta-8,9-Isomer of Avermectin B_1; 91.6 percent purity.

   The test material was evaluated in mutant strains of Salmonella typhimurium (TA97a, TA98, TA100, TA1535) and Escherichia coli (WP2, WP1 uvrA and WP2 uvrA pKM101) at dosages of 0 (DMSO), 10, 30, 100, 300, 1000, and 3000 ug/plate both with and without S9 metabolic activation. Positive controls evaluated were 2-aminoanthracene and hydrazine sulfate. Diagnostic mutagens evaluated were sodium azide, methyl methanesulfonate, daunomycin, and ICI-191 (S. typhimurium strains) and methyl methanesulfonate (E. coli strains).

   Triplicate minimal plates were run for 48 hours with and without metabolic activation at each dose level plus controls (DMSO).

Results - Precipitation occurred at 3000 ug/plate (HDT) with both S. typhimurium strains and E. coli strains. Tables 1 and 2 of the report show that there was neither a doubling nor dose-related increase in revertants, both with and without S-9, tested with the delta-8,9-isomer on the S. typhimurium and E. coli strains. Table 3 of the report shows the significant increases in revertants, in the appropriate strain by the positive controls.

Conclusions - The delta-8,9-isomer is not mutagenic in the Ames assay at dosages ranging from 10 to 3000 ug/plate.

Classification - Acceptable

2. Delta-8,9-Isomer; Oral Developmental Toxicity Study in Rats; MSD No. TT#87-715-0; June 7, 1988; by L. Gordon (MRID No. 407134-03).

Test Material - Delta 8,9-Isomer of Avermectin B_1, L-652,280-000N; Lot No. L-652,280-000N005; purity, 91.6 percent [redacted]

Randomized groups of 25 mated Sprague-Dawley [Crl:CD (SD)BR] female rats were used in the study. The rats were obtained from Charles River Breeding Labs (Wilmington, MA). Each of the 25 rats in each group received 0, 0.25, 0.50, or 1.0 mg/kg bwt/day of test material [redacted] by gavage during days 6 through 17 of gestation. Females were observed for physical signs of toxicity daily and at 1 to 5 hours after the dosing period. Maternal body weight and food consumption were recorded appropriately during the study.

On gestation day 20, the dams were sacrificed and the uterus of each female was examined to determine the reproductive status (pregnant or not pregnant). The number of corpora lutea was counted. Implants were recorded and classified as live fetus, dead fetus, or resorptions.

All fetuses were weighed and examined externally. Visceral evaluation was performed on all externally malformed and dead fetuses and on every third fetus in each litter by dissection. The heads of these fetuses were fixed in Bouin's solution and later examined by free-hand coronal sections. All fetuses were fixed, cleared, and stained with alizarin red for skeletal evaluation.

Gross necropsy examinations were performed on all sacrificed females. All gross lesions were fixed in 10 percent neutral buffered formalin and examined microscopically.

Statistical evaluation was conducted. Results were considered statistically significant at $p < 0.05$.

Results - There were no deaths or abortions during the study. The text states that "Two female rats in the 0.25 mg/kg/day group had alopecia and another rat in this group had chromodacryorrhea" [end of quote]. These toxic signs were not dose-related and were not considered compound-related.

Maternal body weight was increased from days 6 to 18 and 6 to 20 of the gestation period for treated groups as shown below.

Average Maternal Body Weight Gains[a]

	Dose (mg/kg/day)			
Gestational Period	0	0.25	0.50	1.0
No. of Litters	25	24	25	25
Day 6 to 18	79	83	86*	86*
Day 18 to 20	34	33	35	34
Day 6 to 20	113	116	122*	120

[a]grams
*$p < 0.05$

As can be noted from the above maternal body weight data, a statistically significant increase in average body weight gain was observed in the 0.5 and 1.0 mg/kg/day groups during days 6 to 18 and in the 0.5 mg/kg/day group during days 6 to 20.

The increased body weight findings are considered compound-related since similar findings have also been observed in other rat teratology and reproduction studies on Abamectin at similar dosage levels. However, this finding is not considered an adverse effect.

Average maternal food consumption (grams/day) was comparable between control and treated groups during the study.

With respect to reproductive data, the percent preimplantation loss (litter mean) was 7.9, 13.4, 6.2, and 6.6 for the control, low-, mid-, and high-dose groups, respectively.

The increase at the low dose was statistically significant. However, this finding is not considered compound-related, since the increase was not dose-related.

The number of implants per pregnant female, resorptions and dead fetuses (there were no dead fetuses) were comparable between control and treated groups.

Live fetuses per pregnant female were comparable between control and treated groups.

Live fetal weight (grams, litter mean) was increased in the low-, mid-, and high-dose groups in comparison to controls. These differences were 3.57, 3.66, 3.62. and 3.68 g. These findings were not dose-related nor statistically significant and were not considered compound-related.

There were no compound-related external malformations or variations between control and treated groups. The findings are presented below:

	Dose (mg/kg/day)			
	0	0.25	0.50	1.0
No. Fetuses Examined	354	332	374	352
Clubbed forefoot (M)	0	0	0	1
Hind limb rotation (M)	0	0	1	0
Hematoma (V)	1	0	1	0

(M) = Malformation
(V) = Variation

Additionally, there were no compound-related malformations and variations in the visceral and skeletal findings. These findings are shown below:

	Dose (mg/kg/day)			
Fetal Visceral Results	0	0.25	0.50	1.0
No. Fetuses Examined	110	101	116	110
Abnormal origin Subc. Art. (M)	1	0	0	0
Situs inversus (M)	0	0	0	1
Innominate variation (V)	0	0	0	1
Azygos vein variation (V)	2	0	0	0
Distended ureter (V)	1	0	1	1

(M) = Malformation
(V) = Variation

	Dose (mg/kg/day)			
Fetal Skeletal Findings	0	0.25	0.50	1.0
No. Fetuses Examined	354	332	374	352
Missing vertebrae (M)	2[1,2]	3[1,2,3]	1	1[1]
Hypoplastic rib (M)	7[1,2]	7[1,2,3]	10	8[2]
Lumbar rib (V)	11	6	5	8
Pelvic bone variation (V)	0	1	0	0

(M) = Malformation; (V) = Variation
Identical superscripts within the same column denote malformations from the same fetus.

The incidences in the number of litters with fetal delayed ossification sites were comparable between control and treated groups. The incidences in the affected litters were 20, 18, 21, and 21 in the control, low-, mid-, and high-dose groups, respectively.

The findings for fetuses are shown below:

	Dose (mg/kg/day)			
	0	0.25	0.50	1.0
No. Fetuses Examined	354	332	374	352
Sites of Incomplete Ossification				
Cervical vertebrae	1	1	3	2
Thoracic vertebrae	6	8	9	5
Lumbar vertebrae	0	0	0	1
Sacral vertebrae	2	0	0	0
Skull Bone	1	4	1	1
Rib	2	1	4	0
Sternebrae	87	44	70	52
Pelvic bone	0	3	2	2

Conclusion - Maternal weight gain increases occurred in the treated groups in comparison to controls at days 6 to 18 and days 6 to 20 of gestation. Compared to the control group, body weight gain in the 0.5 and 1.0 mg/kg/day groups was statistically significantly increased. However, body weight gain was not considered an adverse effect.

The comparison of control and treated fetuses (and litters) did not show any compound-related effects. The NOEL for developmental toxicity is 1.0 mg/kg/day (HDT). The NOEL for maternal toxicity is also 1.0 mg/kg/day (HDT).

Classification - Core-Minimum (no adverse toxicological effects observed at highest dosage level tested).

3. Delta-8,9-Isomer; Single Generation Study in Rats; MSD No. TT#87-7160; June 7, 1988; by L. Gordon (MRID No. 407134-04).

Test Material - Delta-8,9-Isomer of Avermectin B1; L-652,280-000N; Lot No. L-652,280-000N005; purity 91.6 percent.

Randomized groups of 20 mated Sprague-Dawley [Crl:CD (SD)BR] female rats were used in the study. The rats were obtained from Charles River Breeding Labs [redacted] (Wilmington, MA). The dosages were control [redacted] 0.06, 0.12, and 0.40 mg/kg bwt/day. The period of dosing for the mated females was 15 days prior to

cohabitation, during cohabitation, throughout gestation, and through Day 20 of lactation. The dosing was by gavage and the volume was 5 mL/kg based on most recent body weight.

Parameters evaluated in F_0 dams included toxic signs, body weight, and food consumption. The following reproductive parameters were evaluated:

a. Mating performance;

b. Mating index;

c. Percent mated;

d. Fecundity index;

e. Length of gestation;

f. Percent of live pups;

g. Dead pups; and

h. Live pup weight at days 0, 7, 14, and 21.

All pups found dead were given a limited visceral examination of the trachea and esophagus to look for bedding material. Remaining pups were sacrificed on day 21 of lactation. Necropsy examination of pups was limited to the eyes. Eyes were preserved and examined microscopically.

Females which did not deliver any pups were killed on day 24 of presumed gestation and were given a gross necropsy. The remaining maternal dams were killed on day 21 to 23 of lactation, given a gross necropsy and metrial glands were counted and recorded. Statistical analyses were performed and results were considered to be statistically significant if $p \leq 0.05$.

Results - There were no deaths on abortions in any of the treated or control dams. The number of females with live pups was 18, 18, 17, and 18 for the control, low-, mid-, and high-dose groups, respectively. At the mid-dose, animal 87-3006 was sacrificed pregnant and had only one dead pup and one metrial gland.

The mean day of mating of the females was 3.0, 2.0, 2.1, and 3.0 for the control, low-, mid-, and high-dose groups, respectively.

There were no compound-related effects in mating index, percent mated, fecundity index, or length of gestation for any treated group in comparison to control females.

There were no compound-related effects in female body weight and food consumption for the premating period, gestational period or lactational period. Additionally, no remarkable findings were noted at necropsy for maternal animals.

There were no compound-related effects for implants per female. With respect to the percent postimplantation survival, the litter means were 94.6, 94.1, 84.9, and 94.2 for the control, low-, mid-, and high-dose groups, respectively.

The statistically significant decrease in the mid-dose in comparison to controls (84.9 versus 94.6%) was not considered compound-related. The decrease was largely due to one female (87-3006) which had only one dead pup on day 0 and one metrial gland. Additionally, the high-dose group did not show this effect.

The total number of live pups on day 0 was 254, 242, 218, and 245 for the control, low-, mid-, and high-dose groups, respectively. The total number of dead pups on day 0 was 6, 4, 5, and 1 for the control, low-, mid, and high-dose groups, respectively.

There were no compound-related effects on live pups per litter or on live pups after culling to eight pups per litter on postnatal day 3.

There were no compound-related effects on pup weight on postnatal days 0, 7, 14, and 21. The day 21 pup weights (g) for males and females are shown below:

Pup Body	Dose (mg/kg/day)			
Weight (day 21)	0	0.06	0.12	0.40
Males (Live pups)	51.1	52.4	51.1	52.3
Females (Live pups)	48.8	49.1	48.9	50.0

With respect to external examination, which was performed on all pups, there were no compound-related effects. One unidentified mid-dose pup had anencephaly and ethmocephaly at birth. One pup in the low dose had hydrocephaly.

Gross necropsy of pups found dead during lactation showed the presence of bedding material in the esophagus/trachea region of some pups.

Microscopic examination of the eyes of pups after lactation did not reveal any compound-related effects. The distribution of the most frequently observed ocular lesions is shown below:

Dose (mg/kg/day)	0		0.06		0.12		0.40	
	M	F	M	F	M	F	M	F
No. Examined	73	69	75	69	63	71	70	72
Eye								
Retina, middle anomaly	3	6	4	7	2	8	6	3
Retina, peripheral anomaly	8	12	6	6	6	5	12	10

Conclusion - The NOEL for the one-generation rat reproduction study was 0.40 mg/kg/day (HDT).

Classification - Supplementary (male rats were not tested).

4. L-930,406 (Polar Degradates from Thin Film Dish Photolysis): Microbial Mutagenesis Assay (Ames); (MSD No. TT#87-8047 and TT#87-8058; June 7, 1988; by L. Gordon (MRID No. 407134-05).

Test Material - L-930,406-000N001; polar degradates from a thin film dish photolysis reaction of MK-0936 (Avemectin B_1).

The test material was evaluated in mutant strains of Salmonella typhimurium (TA97a, TA98, TA100, and TA1535) and Escherichia coli (WP2, WP2 uvrA, WP2 uvrA pKM101) at dosages of 0 (DMSO), 100, 300, 1000, 3000, and 10,000 ug/plate both with and without S-9 metabolic activation. Positive controls evaluated were 2-aminoanthracene (S. typhimurium) and hydrazine sulfate (E. coli). Diagnostic mutagens evaluated were sodium azide, methyl methane sulfonate, daunomycin, and ICI-91 (S. typhimurium strains) and methyl methane sulfonate (E. coli strains).

Triplicate minimal plates were run for 48 hours both with and without metabolic activation at each dose level plus controls.

Results - Precipitation of the test material occurred at 10,000 ug/plate (HDT) with all S. typhimurium and E. coli strains both with and without S-9 activation. There were no compound-related increases in revertants at any dosage level in comparison to controls both with and without activation for all of the S. typhimurium and E. coli strains. The positive controls produced increased numbers of revertants (more than double) in the appropriate bacterial strains except for 2-aminoanthracene with metabolic activation. Therefore, a repeat study (TT#87-8058) was performed with metabolic activation only.

The results of the repeat study showed that the test material did not produce a doubling or dose-related increase in revertants in any strains of S. typhimurium or E. coli.

The positive controls showed dose-related increases which were more than double the controls for S. typhimurium and E. coli strains. In contrast to the first assay, 2-aminoanthracene produced dose-related doubling increases with S-9 activation in the appropriate strains.

Conclusion - The polar degradates derived from a thin film dish photolysis reaction were not mutagenic in the Ames assay at dosages ranging from 100 to 10,000 ug/plate.

Classification - Acceptable

5. L-930,406 (Polar Degradates from Thin Film Dish Photolysis); Oral Developmental Toxicity Study in Mice; MSD No. TT#87-717-0; June 7, 1988; by L. Gordon (MRID No. 407134-06).

Test Material - L-930,406-00N001; polar degradates from a thin film dish photolysis reaction of MK-0936 (Avermectin B_1).

Randomized groups of 25 mated female CF_1 (Crl:CF_1BR) mice were used in the study. The mice were obtained from Charles River (Portage, MI). Each of the 25 mice of the treatment groups received 0, 0.25, 0.50, or 1.0 mg/kg bwt/day of test material by gavage during days 6 through 15 of gestation. The dosing volume was 10 mL/kg/day of 0.5% aqueous methylcellulose. Females were observed for toxic signs once daily on days 0 through 17 of gestation, and for 1 to 5 hours post-dosing on days 6 through 15.

Body weight and food consumption were recorded appropriately. On gestation day 17, females were sacrificed and the uterus of each female was examined to determine the reproductive status (pregnant or not pregnant). The numbers of live and dead fetuses and resorptions were recorded. All fetuses were weighed and examined externally. Visceral evaluation was performed on all externally malformed or dead fetuses and on every third fetus in each litter by dissection. The heads of these fetuses were fixed in Bouin's solution and later examined by free hand coronal sections. All fetuses were fixed, cleared, and stained with alizarin red for skeletal examination.

Following laparotomy, all females were given a gross necropsy examination and discarded.

Statistical analyses were performed with $p < 0.05$ being significant.

Results - There were no deaths or abortions in any group including controls. There were no compound-related toxic signs. There were no compound-related effects in maternal body weight and food consumption. The number of live, pregnant females were 21, 23, 22, and 24 for the control low-, mid-, and high-dose groups, respectively. The number of implants per pregnant female were 13.2, 12.8, 13.4, and 13.4 for the control, low-, mid-, and high-dose groups, respectively. Additionally, there were no compound-related effects in the number of resorptions and dead fetuses per implant (litter mean).

The ratio of live fetuses/pregnant female was 11.3, 10.8, 12.0, and 12.4 in the control, low-, mid-, and high-dose levels, respectively. Fetal body weight was unaffected by treatment. The live fetal weights were 0.92, 0.96, 0.93, and 0.95 g, for the control, low-, mid-, and high-dose groups, respectively.

With respect to external malformations, cleft palate occurred in 1, 2, 0, and 2 litters and in 1, 2, 0, and 3 fetuses in the control, low-, mid-, and high-dose groups, respectively. The incidence of cleft palate in this study is not considered compound-related, since this malformation did not occur in a statistically significant trend ($p > 0.05$) and frequently occurs in CF_1 mice at similar incidences. Historical control data supporting this statement were presented in the study report.

Other external malformations occurred as single fetal incidences in either control or treated groups. These malformations were exencephaly (one control, one mid-dose, and one high-dose), open eyelid (one control, one mid-dose), gastroschisis (one dead mid-dose fetus), and polysyndactyly (one control fetus).

Visceral malformations also occurred as single fetal incidences in either control or treated groups. The occurrence of visceral malformations and variations were not considered compound-related.

The type and number (both litters and fetuses) of skeletal malformations and variations did not occur in a compound-related manner. The most frequently occurring skeletal abnormalities are presented below as reported in the study:

	Dose (mg/kg/day)			
	0	0.25	0.50	1.0
Number of Fetuses Examined	237	249	263	298
Sternebrae malformation (M)	6	6	3	9
Cervical rib (V)	14	13	10	15
Lumbar rib (V)	28	24	15	19

(M) = Malformation
(V) = Variation

The number of litters and fetuses with sites of incomplete ossification did not occur in a compound-related manner as presented below:

Summary of Fetal Ossification Data

	Dose (mg/kg/day)			
	0	0.25	0.50	1.0
Number of Fetuses Examined	237	249	263	298
Incomplete Ossification				
Cervical vertebrae	1	0	1	2
Sacral vertebrae	0	0	1	0
Skull bone	2	5	3	5
Sternebrae	5	3	4	4
Pelvic bone	1	0	0	1
Number of Litters Examined	21	23	22	24
No. with incomplete ossification	5	6	4	6

Conclusion - The polar degradates of Avermectin B_1 did not show any evidence of maternal or developmental toxicity. The NOEL for maternal toxicity is 1.0 mg/kg/day (HDT). The NOEL for developmental toxicity was also 1.0 mg/kg/day (HDT).

Classification - Core-Minimum (no adverse toxicological effects observed at highest dosage level tested).

Attachments

R:53500:Dykstra:C.Disk:KENCO:2/27/89:AS:VO:EK:AS
R:56507:Dykstra:C.Disk:KENCO:3/8/89:AS:vo:rw:EK:CL

REVIEWER
007080
Attachment

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 2 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Avermectin - Meeting With Registrant (Merck).

Caswell No.: 638B

FROM: William Dykstra
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: George T. LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Edwin F. Budd, Section Head
Review Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

On August 3, 1987, a meeting was held between representatives of Merck, Toxicology Branch (TB) (Dr. Farber, E. Budd, and W. Dykstra), and A. Heyward of PM Team 15 from Registration Division to discuss additional data requirements for permanent tolerances on citrus with Avermectin (Abamectin).

1. For the delta-6,9-isomer, the following data were requested:

   a. Ames assay (with and without activation); depending on the results of this study, additional mutagenicity data may be required. Before making a final decision on the need for additional mutagenicity testing, the results of the chronic feeding oncogenicity studies on avermectin will also be considered. These studies are presently under review in TB.

16

c. Teratology study in rats; the highest dosage level tested in this study is to be a "small" multiple (e.g., 5-10X) of the maternotoxic/ developmental toxic effect level observed in the mouse teratology studies with this isomer. The need for a teratology study in a third species (rabbits) will be decided after the rat study is completed. It was noted by Merck that the delta-8,9-isomer would be extremely difficult to isolate and purify in the volume required for a rabbit teratology study.

d. Reproduction study in rats; it was mutually agreed that a full multigeneration study was not necessary and that some type of "shortened or abbreviated" study would suffice. Merck will devise a novel protocol for this study and submit it to EPA for approval prior to initiating the study. It was also agreed that this study may be designed in such a way as to incorporate into it the rat teratology study requested in b. above (at the discretion of Merck).

e. EB will retract its previous request for a 90-day feeding study in rats on the delta-8,9-isomer, provided that new information, as it becomes available, does not raise significant new concerns with respect to the potential oral toxicity of the isomer.

2. For the polar degradates, the following data were requested:

a. Ames assay (with and without activation); depending on the results of this study, additional mutagenicity data may be required.

b. Single-group multiple-dose test at 0.5 mg/kg/day in pregnant CF-1 mice to evaluate maternotoxicity and teratogenicity.

3. Ancillary information:

a. The registrant will submit additional data on reproduction, mutagenicity, etc., with Avermectin and/or Ivermectin.

b. Slides of eyes of weanlings and adults in the two-generation rat reproduction study with Avermectin were submitted. The slides have been sent to EPL for evaluation.