



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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: March 19, 1982

SUBJECT: 618-EUP-RN; MK-936; Avermectin B₁ for control of
fire ants CASWELL#63AB; Acc.#246894-895

FROM: William Dykstra, Toxicologist
Toxicology Branch/HED (TS-769)

TO: George LaRocca (15)
Registration Division (TS-767)
and
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

WJD *fdc*
3/19/82
b/csp

Recommendations:

- 1) The EUP program is not toxicologically supported.
- 2) The registrant is required to determine the exposures to the active ingredient for applicators who apply the bait by hand, adults and children who come into contact with treated soil, and livestock which consume the treated grass.
- 3) Toxicology Branch requires that the rat and rabbit teratology studies which were noted in the letter of 1/29/81 to Mr. Herb Harrison be submitted.
- 4) In teratology studies TT#76-723-0 and TT#76-723-3 and TT#77-705-0, the registrant is required to provide a rationale as to why the 0.1 and 0.2 mg/kg/day groups were not examined for visceral and skeletal malformations and variations or submit these data.
- 5) In the teratology study TT#76-705-0, it appears in Table 3 that 5 fetuses had ablepharia at 0.8 mg/kg/day rather than 3 fetuses in one litter as stated in the results section of the report. Additionally, it is uncertain how many litters were affected.

- 6) Is compound C-076 (B₂) a component of Avermectin B₁?
- 7) The oral reproduction studies in rats are considered to be teratology studies with postnatal evaluations.
- 8) The oral toxicity studies in dogs and rats are acceptable as supplementary data only.

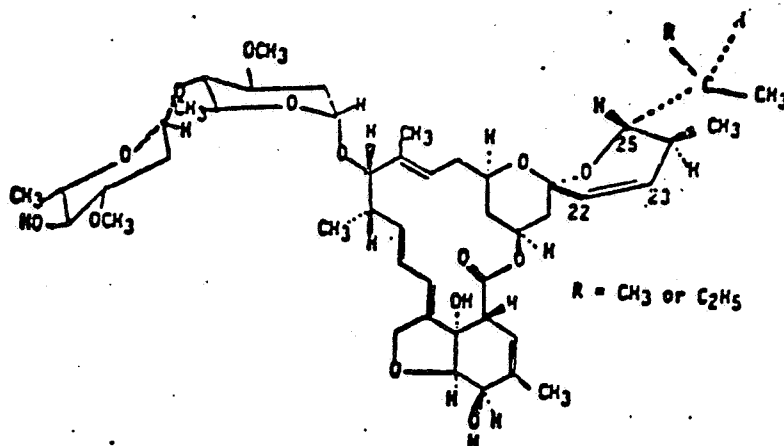
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Introduction

The Merck Sharp & Dohme Research Laboratories have recently discovered the broad spectrum agricultural pesticidal activity of the avermectins, a novel class of macrocyclic lactones isolated from the soil organism *Streptomyces avermitilis*. In greenhouse and field studies these streptomycete-derived natural products have demonstrated high potencies against phytophagous mites and insect pests in several orders. Behavioral observations and mode of action studies indicate that the avermectins mechanism of toxicity to arthropods is fundamentally different from that associated with current natural and synthetic pesticides. Thus, with the avermectins we have uncovered a new toxicophore with excellent potential for use in agriculture.

Chemistry

The chemical structures of the avermectin family of compounds, which contain a 16-membered lactone ring connected at the 13-position to the disaccharide unit α -L-oleandrosyl- α -L-oleandrosyl, represent distinct and novel pesticidal chemicals. Avermectin B₁ which has been selected for development as an



Empirical Formula

(R=C₂H₅) C₄₈H₇₂O₁₄

(R=CH₃) C₄₇H₇₀O₁₄

Molecular Weight

872.58

858.56

acaricide/insecticide is produced by fermentation and is a mixture of two compounds belonging to the avermectin family. The chemical names of the two compounds are avermectin B_{1a} (R=C₂H₅) and avermectin B_{1b} (R=CH₃). Avermectin B₁ is defined as the product that contains at least 80% of the compound in which R in the above structure is the ethyl group and less than 20% of the compound in which R is the methyl group. These structures differ chemically by only one

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methylene (CH₂) unit at the 25-carbon position and studies in our laboratories have clearly demonstrated that the individual components have very similar biological and toxicological properties and, for all practical purposes, can be considered equivalent.

Avermectin B₁ is a yellowish white crystalline powder which is stable when stored under ambient conditions. The compound is very insoluble in water (≤ 5 ug/ml), but readily soluble in organic solvents such as alcohols, aromatic hydrocarbons, acetone, and methylene chlorido. The material is optically active and has a specific rotation, $[\alpha]_D^{27}$ of $+55 \pm 20$ (chloroform). The ultraviolet absorption spectrum in methanol is characterized by a maximum at 245 nm (63185).

Pesticidal Activity

Avermectin B₁ has demonstrated potent broad spectrum miticidal and insecticidal activity in greenhouse and field trials. The product is most effective when ingested by the target pest although there is evidence that uptake by contact action may also contribute in a significant way to overall activity in some insect species. Avermectin B₁ has demonstrated good residual activity to foliar feeding insects and mites. The compound is a slow-acting toxicant. As much as 72 to 96 hours may be required following treatment to observe maximum mortality although insect inactivation (paralysis) occurs shortly after exposure to the pesticide spray. Avermectin B₁ is effective against all motile feeding stages of mites and insects, but has not shown ovicidal action at rates up to 12X the projected field use rate.

Table I below provides examples of field rates projected to be effective in providing commercially acceptable pest control and yield improvements for some crops and pest applications.

TABLE I

<u>Crop</u>	<u>Pest</u>	<u>Estimated Rate</u>	
		<u>(lb ai/acre)</u>	<u>(g ai/acre)</u>
Citrus	citrus rust mite	0.01-0.02	4.5-9.0
Citrus	citrus red mite	0.02-0.04	9.0-18.0
Cotton	two-spotted spider mite	0.01-0.02	4.5-9.0
Apples	European red mite	0.02-0.04	9.0-18.0
Potatoes	Colorado potato beetle	0.005-0.01	2.25-4.5
	Red imported fire ant	50 mg/acre	0.05

The high potency and consequently low application rates of avermectin B₁ should be pointed out. As an acaricide it appears to be effective at field rates 1/50 to 1/300 that of current products while as an insecticide for the Colorado potato beetle avermectin B₁ displays a possible 10 to 100-fold potency advantage over the available standards. In fire ant studies by USDA researchers it was found that B₁ at about 50 mg/acre in a bait may be sufficient to provide effective control of this important pest.

Review:

1. Acute Oral Toxicity in Mice and Rats (MSD; No number; No date)

Test Material: C-076 (B_{1a}) (L-676, 895-00P26)

The oral LD₅₀ values are shown below:

<u>Species</u>	<u>Strain</u>	<u>Sex</u>	<u>LD₅₀ (95% con. lim.) mg/kg</u>
mouse	CF ₁	F	22.2 (15.7 - 31.5)
mouse	CF ₁	F	23.8 (14.3 - 39.6)
mouse	CF ₁	F	13.7 (7.3 - 25.3)
mouse	CF ₁	F	18.3 (10.1 - 33.2)
mouse	CD	F	17.4 (12.1 - 25.1)
mouse	ICR (MSD)	F	18.7 (12.9 - 27.1)
Rat (young)	CRCD	M	10.6 (7.7 - 14.5)
Rat (young)	CRCD	F	11.3 (7.5 - 17.1)
Rat (weanling)	CRCD	M,F	1.5 (1.1 - 2.2)

Toxic Signs: In mice; It was reported that mice had ataxia, bradypnea, tremors, loss of righting activity.

In rats; It was reported that rats had tremors, decreased activity, ataxia, loss of righting reflex, chromodacryorrhea, chromorhinorrhea.

Body Weight: It was reported that survivors had normal body weight gain.

Necropsy: Not reported.

Toxicity Category I: DANGE

Classification: Supplementary Data (a) Details of each study not provided.

2. Ames Assay (Bacterial Mutagenic Study) (MSD Report No. TT#76-8052; No date)

Test Material: C-076 (B_{1a}) L-676, 895

The test material was tested twice. The first assay employed 1, 10 and 100 ug/plate and the second assay used 20, 200 and 2000 ug/plate.

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The strains of Salmonella typhimurium used were TA1537, TA100, TA98 and TA92. The tests were conducted both with and without metabolic activation from the microsomal fraction of rat liver.

A positive control, identified as MK-436 was also tested.

Results: The test material was not mutagenic under the conditions of the study.

Classification: Acceptable

3. Oral Range-Finding Studies (MSD Report No. TT#76-723-1, TT#76-723-2; No date)

Test Material: C-076 (B_{1a}) L-676, 896, P10); C-076 (B₂) L-676, 897, Lot G05

In two range-finding studies pregnant female mice (CF strain) were assigned to 16 groups of 5 mice each. The groups received either C-076(B_{1a}) or C-076(B₂) daily from days 6 to 15 of gestation.

In the first study, the dosage levels were 0.1, 0.25, and 0.5 mg/kg/day of C-076(B_{1a}) and 0.1, 0.5 and 0.75 mg/kg/day of C-076 (B₂). The dosage levels in the second study were 1.0, 2.0, 4.0, 6.0 and 8.0 of either C-076 (B_{1a}) or C-076 (B₂).

An additional group of 5 CF₁ strain mice served as a control for each study and received the vehicle (0.5% methylcellulose) in the same volume (10 ml/kg) and dosing regimen as the treatment groups. On day 18 of gestation, the mice were weighed, sacrificed and their reproductive indices recorded.

Results:

Administration of C-076 (B_{1a}) resulted in death in 1 of 5 mice in each of the 0.1, 0.25, and 1.0 mg/kg/day groups, and 3 of 5 mice at 8.0 mg/kg/day group.

Although there were no deaths, 2 of 5 mice at 4.0 mg/kg/day had tremors after receiving the first dose.

Average body weight gains in surviving mice during gestation were not affected in the 0.1, 0.25 and 0.5 mg/kg/day groups. Decreased body weight gain was noted in the 1.0 mg/kg/day group. Significant decreases in body weight were noted in the 2.0, 4.0, 6.0 and 8.0 mg/kg/day groups.

There were no deaths in mice given 0.1, 0.5 or 0.75 mg/kg/day of C-076 (B₂). Deaths occurred in 1 of 5 mice in each of the 1.0, 2.0 and 8.0 mg/kg/day groups after one or two doses.

Average weight gains were unaffected in the 0.1, 0.5 and 0.75 mg/kg/day groups. Decreased weight gain was observed at 1.0, 2.0, 4.0, 6.0 and 8.0 mg/kg/day.

Classification: Supplementary Data

4. Oral Teratogenic Studies (MSD Report No. TT#76-723-0, TT#76-723-3; No date)

In the first teratogenic study (TT#76-723-0) eight groups of 10 pregnant CF₁ strain mice each received either C-076 (B_{1a}) or C-076 (B₂) at dosages of 0.1, 0.2, 0.4 or 0.8 mg/kg/day from days 6 to 15 of gestation.

Two control groups received the vehicle, sesame oil, in the same volume (10 ml/kg) and dosing regimen as the treatment group.

A second teratogenic study (TT#76-723-3) was conducted as a replicate except that there 15 CF₁ mice/group (8 treatment and 2 controls).

The mice were weighed on day 18 of gestation in both studies, were sacrificed, and their reproductive status recorded. All fetuses were weighed.

Visceral examination was performed on every third fetus in each litter from the control I, control II, 0.4 and 0.8 mg/kg/day by serial section. All skeletons of fetuses from control I, control II, 0.4 and 0.8 mg/kg/day were stained with alizarin red and examined for anomalies and variations.

Results:

C-076 (B_{1a})

One, 3, 6, and 8 mice died in the 0.1, 0.2, 0.4 and 0.8 mg/kg/day groups, respectively.

Average weight gains of surviving mice was unaffected in each group. Implantations, resorptions, live and dead fetuses and fetal weight was unaffected by treatment.

There was a dose-related increase in cleft palates in the 0.4 and 0.8 mg/kg/day group treated with C-076 (B_{1a}). There were 5 fetuses in two litters and 10 fetuses in four litters affected at 0.4 and 0.8 mg/kg/day, respectively.

All of the other visceral and skeletal malformations and variations were not considered treatment-related.

C-076 (B₂)

Two, 3, 5, and 5 mice died in the 0.1, 0.2, 0.4, and 0.8 mg/kg/day groups. Average weight gain of surviving mice was unaffected by treatment. The average number of implantations, resorptions, live and dead fetuses, and fetal weight was unaffected by treatment. There was a dose-related increase in cleft palates in the 0.2, 0.4, and 0.8 mg/kg/day group. There were 2 fetuses in two litters, five fetuses in 3 litters and 10 fetuses in 5 litters with cleft palate in the 0.2, 0.4, and 0.8 mg/kg/day groups, respectively.

All of the the other visceral and skeletal malformations and variations were not considered treatment-related.

Conclusion: C-076 (B_{1a}) was teratogenic at 0.4 and 0.8 mg/kg/day. C-076 (B₂) was teratogenic 0.2, 0.4 and 0.8 mg/kg/day. No NOEL was established for maternal toxicity. Both compounds produced cleft palate.

Classification: Supplementary Data (a) Fetuses at 0.1 and 0.2 mg/kg/day were not examined.

5. Oral Teratogenic Evaluation in Mice with C-076 (B_{1a}) (MSD Report No. TT#77-705-0; No date)

Test Material: C-076 (B_{1a}); L-767,895; Lot P20

Four groups of 20 pregnant CF₁ strain mice each were given the test material in sesame oil once daily by gavage from days 6 to 15 of gestation at doses of 0.1, 0.2, 0.4 and 0.8 mg/kg/day.

Two additional groups of 20 pregnant mice each served as vehicle controls. Body weights were recorded on day 1 and on alternate days from day 6 to 16 of gestation with doses based on the most recent body weight. On day 18 of gestation, the mice were weighed, sacrificed and their reproductive status recorded. Fetuses were weighed and examined externally.

Visceral examination by serial dissection was performed on every third fetus in each litter from control I, control II, and 0.4 and 0.8 mg/kg/day groups.

All skeletons of fetuses from the control I, control II, and 0.4 and 0.8 mg/kg/day groups were stained with alizarin red and examined for malformations and variations. All externally malformed fetuses and dead fetuses were examined for visceral and skeletal malformations and variations.

Results:

One, 2 and 2 mice died in the 0.1, 0.4 and 0.8 mg/kg/day groups, respectively.

Average maternal weight gain was unaffected by treatment. The number of implantations, resorptions, and live and dead fetuses per litter was unaffected by treatment. Slight decreases in average fetal weight per litter occurred at 0.1 and 0.8 mg/kg/day groups. The decrease at 0.1 mg/kg/day was significant. Cleft palate occurred in 2 litters each in 4 and 5 fetuses of the 0.4 and 0.8 mg/kg/day groups, respectively.

Five fetuses from the 0.8 mg/kg/day group had ablepharia whereas only 1 fetus from control had this malformation. These terata may be treatment related. All of the other visceral and skeletal malformations and variations were not considered treatment-related.

Conclusion:

C-076 (B_{1a}) was teratogenic at 0.4 and 0.8 mg/kg/day groups. A NOEL for maternal toxicity was not established since at 0.1 mg/kg/day, toxicity and death occurred in one mouse.

Classification: Supplementary Data (a) fetuses at 0.1 and 0.2 mg/kg/day were not examined.

6. Ten-Day Oral Toxicity Study in Pregnant Mice (MSD Report No. TT#77-717-1; No Date)

Test Material: C-076 (B_{1a}); L-676,895; Lot P34

The test material was given orally by gavage to four groups of 20 pregnant CF₁ strain mice each from days 6 to 15 of gestation at dosage levels of 0.025, 0.050, 0.075 and 0.10 mg/kg/day.

One additional group of 20 pregnant mice served as a control and received the vehicle, sesame oil, in the same volume (10 ml/kg) and dosing schedule as the treated groups.

Results: One mouse died in each of the 0.10 and 0.075 mg/kg/day groups. Toxic signs in the mouse that died at 0.10 mg/kg/day included tremors and coma prior to death after receiving the third dose. Similar whole body muscular tremors were seen in 2 additional mice in this group. The mouse at 0.075 mg/kg/day which was sacrificed was comatose and aborting after receiving 4 doses. No other mice in this group or in the 0.05 or 0.025 mg/kg/day groups displayed any toxic signs. There was no treatment-related effect on average maternal weight gain.

Conclusion: The NOEL for maternal toxicity in 0.05 mg/kg/day.

Classification: Core-Minimum Data

7. Oral Reproduction Study in Rats (MSD Report No. TT#77-706-0; No date)

Test Material: C-076 (B_{1a}) (L-676,895); Lot P20

Three groups of 12 Charles River CD strain rats received oral doses by gavage of the test material as a solution in sesame oil at dosage levels of 0.5, 1.0 and 3.0 mg/kg/day. After five doses the highest dosage was reduced to 1.5 mg/kg/day because of muscular tremors observed in 3 females. Two control groups of 12 rats each received the vehicle in the same volume (5 ml/kg) as the treated rats.

On the fifteenth day of treatment, the females were placed with untreated males of the same strain, and dosing was continued throughout the mating period. Dosing of the females continued through gestation and until day 21 postpartum.

Body weights of the females were recorded on days 1, 8 and 15 of the prebreeding period and on days 1, 7, 15, 17, 19, 21 and 22 of gestation. During lactation weights of all females were taken on days 2, 7, 14 and 21.

On day 1 postpartum the pups were counted, weighed, examined externally, and sexed. Litter size was standardized by random selection where possible to 8 pups, and all excess pups were discarded. Cross fostering of pups within the same dosage group was used if necessary to standardize litter size. Pups were weighed on days 7, 14, and 21, and sexed on day 21. Because of deaths, decreased body weight, and adverse

physical signs observed at weighing on day 7, the litters were examined daily for physical signs beginning on day 7 postpartum. In addition, an examination for eye opening was made daily from day 13 until all pups in a litter had both eyes open.

At the termination of the study all females were sacrificed, and the number of metrial glands counted. In addition, females were sacrificed if they did not successfully mate within a 29-day period; failed to deliver by day 25 gestation; or if all pups died in a litter prior to the completion of the 21-day lactation period. All pups were discarded at the termination of the study.

Results:

No mortality or toxic signs were noted among female rats given 0.5 and 1.0 mg/kg/day during the prebreeding, gestation, or lactation periods.

Three female rats died at the 1.5 mg/kg/day dosage level.

Dosage levels of 0.5 and 1.0 mg/kg/day did not affect adversely the female rats body weight gains during prebreeding, gestation, and lactation. No adverse affect on body weight gain was noted in the surviving rats of the 1.5 mg/kg/day group during the study. The test material had no effect on the breeding, reproductive status or postimplantation survival rate of females at doses of 0.5, 1.0 and 1.5 mg/kg/day.

Pup survival at day 1 in the 0.5 and 1.0 mg/kg/day groups was comparable to controls but was slightly reduced at the 1.5 mg/kg/day level. Between day 1 and 21, pup survival and growth were adversely affected at all dosage levels in a dose-related manner. At day 21, pup survival in the 0.5, 1.0, and 1.5 mg/kg/day groups was 76, 14, and 0 percent, respectively, compared to 98 percent in the pooled controls.

Average pup weights were significantly decreased in a dose-related manner beginning on day 7.

There was a developmental delay of several days in the time to occurrence of eye opening in pups from the 0.5 and 1.0 mg/kg/day groups in comparison to controls.

Conclusion: A NOEL was not established in this study.

Classification: Supplementary Data (a) This study is a teratology study with postnatal evaluation. It is not a reproduction study since males were not treated. (b) Inadequate number of pregnant rats.

8. Oral Reproduction Study in Rats (MSD Report No. #TT77-712-0; No date)

Test Material: C-076 (B_{1a}); L-676,895-00P22

Three groups of 15 female rats each were given orally by gavage dosages of 0.1, 0.2 and 0.4 mg/kg/day of test material. Two control groups of 15 female rats each were given the vehicle, sesame oil, in the same volume (5 ml/kg) as the treated rats.

On the fifteenth day of treatment the females were placed with untreated males of the same strain and dosing was continued throughout the mating period.

Body weights were recorded on days 1, 8, and 15 of the prebreeding period and on days 1, 7, 15, 17, 19, 21, and 22 of gestation. During lactation weights of all females were recorded on days 2, 7, 14, and 21.

On day 1 postpartum the pups were counted, weighed, examined externally, and sexed. Litter size was standardized by random selection where possible to 8 pups, and all excess pups were discarded. Pups were examined daily and weighed on days 7, 14, and 21, and sexed on day 21. In addition, examinations for earflap opening, eye opening, incisor eruption and hair growth were made daily until all pups in the litter exhibited the individual developmental signs.

Results:

No mortality or toxic signs were observed among female rats given 0.1, 0.2 or 0.4 mg/kg/day during the prebreeding, gestation or lactation periods. Body weight gain was not adversely affected during the study at all dosage levels.

There was no effect on mating, reproductive status, average length of gestation, or postimplantation survival rate of females at all dosage levels.

There was no treatment-related pup mortality among dams at any treatment level from postpartum to day 21. No physical signs of toxicity were observed in pups from dams treated with 0.1 mg/kg/day. At 0.2 mg/kg/day 7 of 13 litters had at least 1 pup which showed occasional spastic movements of the limbs between days 6 and 16 postpartum, but these were infrequent, very slight in degree, and were observed on only one or two occasions in each litter affected. Among pups in the 0.4 mg/kg/day dosage level group these spastic movements were more pronounced and often accompanied by muscular tremors

of the entire body. All litters at 0.4 mg/kg/day shown pups with one or both of these signs, and these signs of toxicity persisted to day 23 postpartum in some litters. Hypothermia and a reduced quantity of milk in the stomach were also observed in some pups from high dose litters.

There were no treatment-related effects on the average day 1 pup weight or on the number of live pups per litter on day 1. There was a slight but statistically significant decrease in the number of live pups per litter at 0.4 mg/kg/day compared to pooled controls at day 1.

Average pup weight on days 7, 14, and 21 was unaffected by treatment with 0.1 mg/kg/day. At 0.2 mg/kg/day there was no effect on the average pup weight per litter; however, the average weights of pups from dam 77-0683 were reduced to approximately the same degree as those from the high dose group, and this is considered to be treatment-related. At 0.4 mg/kg/day there were treatment-related, significant decreases in average pup weight compared to controls on days 7, 14, and 21 postpartum.

There were no treatment-related effects on the time to occurrence of ear opening, incisor eruption, hair growth, and eye opening in pups from mothers treated with 0.1 mg/kg/day. At 0.2 mg/kg/day the only significant effect was a decrease in the average time to incisor eruption. At 0.4 mg/kg/day there were significant increases compared to controls in the time to occurrence of ear opening, hair growth, and eye opening, and a significant decrease in the time to incisor eruption. The relationship to treatment of the decrease in the time to incisor eruption at 0.2 and 0.4 mg/kg/day is uncertain but may be the result of a reduced body weight compared to controls.

External, visceral, and skeletal examinations of dead pups from all treatment and control groups revealed no evidence of teratogenicity as there was only one malformation (clubbed rib) found in a 0.4 mg/kg/day pup.

Conclusion: The NOEL for the study is 0.1 mg/kg/day.

Classification: Supplementary (a) This study is a teratology study with postnatal evaluation. It is not a reproduction study since males were not treated. (b) Only dead pups examined (skeletal & visceral).

9. Eighteen-Week Oral Toxicity Study in Dogs (MSD Report No. TT#76-073-0; No date)

Test Material: C-076 (B_{1a})

Three groups of 3 male and 3 female beagle dogs were gavaged daily with 0.5, 2.0, and 8.0 mg/kg/day of the test material in sesame oil. Two additional groups of 3 male and 3 female dogs received tap water and sesame oil, respectively, in the same volumes (1 ml/kg) and dosing schedule.

An additional treatment group which was added on the second day of dosing received 0.25 mg/kg/day in an identical manner.

Dosing of the 8.0 and 2.0 mg/kg/day groups was discontinued after one and three doses, respectively, because of deaths and the deteriorating physical condition of the dogs.

Dosing of one 0.5 mg/kg/day male (76-0254) was suspended for four days in treatment - week 3 to allow the dog's poor physical condition to improve. Dosing of this dog was then resumed and continued until the termination of the study.

All surviving dogs were examined 5 days a week for signs of toxicity with less detailed observations recorded on weekends and holidays. Animals were weighed twice a week during the study. Routine hematologic and serum biochemical determinations were performed in treatment weeks 4, 8, 13, and 17 for dogs in the 0.5, 2.0, and 8.0 mg/kg/day groups and in treatment Weeks 4, 7, 12, and 17 in the 0.25 mg/kg/day. Additional hematologic and serum biochemical studies were performed on 8.0 mg/kg/day dogs in treatment Week 1, and an additional bleeding was done on all dogs in treatment Week 13 for serum electrophoretic analysis. Urinalyses were conducted in treatment Weeks 4, 8, 13, and 18 on dogs in the 0.5, 2.0, and 8.0 mg/kg/day group and in treatment Weeks 4, 7, 12, and 17 for dogs at 0.25 mg/kg/day. There were ophthalmologic examinations of dogs in the 0.5, 2.0, and 8.0 mg/kg/day groups in treatment Weeks 3, 8, 12, and 16, and of 0.25 mg/kg/day dogs in treatment Weeks 3, 8, 11, and 15. Electrocardiograms were recorded in treatment Weeks 2, 3, 8, 12, and 17 for dogs in the 0.25, 0.5, 2.0, and 8.0 mg/kg/day groups.

Additional electrocardiograms were recorded on 8.0 mg/kg/day dogs in treatment Week 1. All surviving dogs were killed and necropsied in treatment Week 18.

Samples of all tissues were preserved in 10 percent neutral buffered formalin, or Zenker's acetic fixative. Hematoxylin- and eosin-stained sections of most tissues were examined from all dogs in the three highest dosage levels, and from dogs in the Control I (tap water) group. Routine examinations included paraffin-embedded sections of left eye and optic nerve, heart, liver, gall bladder, spleen, pancreas, mesenteric lymph node, stomach (2 levels), small and large intestine (3 levels), adrenal, kidney, urinary bladder, thyroid (frequently including parathyroid), lung, thymus, salivary gland, rib bone, bone marrow smear, pituitary, brain, spinal cord, sciatic nerve, testis and prostate (or ovaries and uterus), skeletal muscle, and skin (frequently including mammary gland). Based on the presence of a histomorphologic change seen during extensive histologic examination, sections of rib from dogs in the Control II (sesame oil) and 0.25 mg/kg/day groups were also processed and examined histologically. In those animals where liver and kidney were examined using hematoxylin- and eosin-stained sections, frozen sections of fixed samples were also stained with oil red O, to assess the presence of neutral lipid.

Results:

There were no deaths in the 0.25 mg/kg/day group. One female dog in the 0.5 mg/kg/day group was found dead after receiving 18 doses and treatment-related histologic changes were noted in the liver. Two males and 1 female dogs were found dead in the 2.0 mg/kg/day group. Histologic examination of the liver showed treatment-related effects. At 8.0 mg/kg/day, two male and 1 female dog died after receiving one dose, and treatment-related changes were seen in the liver. No toxic signs were observed in dogs at 0.25 mg/kg/day.

A retarded pupil response to direct light was noted in one 0.5 mg/kg/day dog on treatment day 7. In treatment Week 2 mydriasis or retarded pupil responses to direct light were seen in 4 of 6 dogs at this dosage level. Mydriasis or retarded pupil responses were a consistent finding in treatment Week 3. No mydriasis was observed beyond treatment Week 4 and only 1 dog showed a retarded pupil response in treatment Week 5. Three of 6 dogs at 0.5 mg/kg/day had whole body muscular tremors on at least one occasion in treatment Weeks 2 and 3. One dog at this dosage level exhibited slight whole body muscular tremors in treatment Week 2. Ataxia, muscular tremors, ptyalism, and mydriasis or retarded pupil response were observed in treatment Week 2 in 1 dog, and these same toxic signs, with the exception of ataxia, also occurred in treatment Week 3. These toxic signs persisted until the second

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day of four-day drug withdrawal. After allowing the dog's condition to improve, dosing was resumed and no further signs of toxicity were observed. In treatment Week 3 dog 76-0265 F displayed ataxia with whole body muscular tremors and ptialism and was found dead on day 19.

Ataxia, ptialism, mydriasis, emesis, and anorexia were consistent findings in all dogs at 2.0 mg/kg/day by day 3 of dosing. In addition, 2 of 3 dogs found dead in this group after the third dose had displayed whole body muscular tremors three to seven hours postdosing, and 1 of the 3 had tonic convulsions six to seven hours after the third dose. One other dog (76-0246 M), which was not observed to have tremors, had tonic convulsions six to seven hours after the third dose. None of the physical signs, other than mydriasis and anorexia, persisted more than 24 hours after the third and final dose. Anorexia continued in some dogs up to 48 hours after dosing was suspended, and mydriasis persisted in 1 dog up to 72 hours after having received the third and final dose.

Ptyalism, emesis, ataxia and/or whole body muscular tremors were observed in the 8.0 mg/kg/day dosage group within three hours of the first dose. Tremors progressed to tonic convulsions within three to five hours postdosing in 4 of 6 dogs, and 2 of 6 dogs died within four hours of dosing. Mydriasis was observed 24 hours postdosing in the surviving dogs in this dosage group. Twenty-four hours postdosing, dog 76-0246 M was prostrate, had ptialism, and whole body tremors. This dog received 200 cc of 2.5 percent dextrose in saline subcutaneously infused twice daily for four days until its physical condition improved. Periodic tremors were observed in this dog for up to four days following the initial dose. The dogs were observed to have a bilateral crusty exudate around the eyes on day 3 of the study, apparently resulting from abrasions incurred by the floor of the cage during tremors and/or convulsions. A corneal opacity was observed in the left eye of this dog on day 6, and mydriasis was observed for the final time five days postdosing.

Body weights of dogs in the 0.25 mg/kg/day group were comparable to those of the control group.

There were treatment-related decreases in body weight in the 0.5 and surviving dogs at 2.0 mg/kg/day groups.

No treatment-related changes in body weight were noted in surviving dogs at 8.0 mg/kg/day, although dogs at this dosage level received only one dose.

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There were no treatment-related effects in hematology or clinical chemistry in any group.

Urinalysis and ophthalmologic examination did not reveal any treatment-related effects.

Electrocardiographic recordings of dogs in the 0.25, 0.5, and 2.0 mg/kg/day dosage groups were comparable to those of the controls.

In treatment Week 1 dogs in the 8.0 mg/kg/day dosage group showed bradycardia (range 32 to 109) and an increase in the QT interval (range 0.24 to 0.28 sec.) compared to pretest or treatment Week 4 values. Bradycardia and QT segment lengthening were most apparent in 3 dogs which later were found dead. Subsequent electrocardiographic recordings in this dosage group were comparable to those of the control groups.

There were no treatment-related changes in absolute or relative organ weights.

Three dogs at 8.0 mg/kg/day, and at 2.0 mg/kg/day, and 1 dog at 0.5 mg/kg/day died prior to scheduled terminal autopsy. Consistent and significant changes were limited to the hepatobiliary system in all but 1 dog. Diffuse vacuolation of hepatocytes was observed in 3 dogs at 8.0 mg/kg/day, 2 at 2.0 mg/kg/day, and in the 1 dog at 0.5 mg/kg/day. Oil red O staining did not establish a correlation between vacuoles and fat content. Edema of the gall bladder, primarily in the subserosal connective tissue, was noted grossly in 3 dogs (2 at 8.0 mg/kg/day and 1 at 2.0 mg/kg/day); microscopically, the edema was confirmed in these dogs, and was noted in an additional dog at 2.0 mg/kg/day. The remaining gross and microscopic changes noted in these animals were scattered, of low incidence, and were of the type commonly seen in untreated dogs.

There were no treatment-related histologic changes in tissues of animals that survived the experiment.

Conclusion: The NOEL is considered to be the 0.25 mg/kg/day group.

Classification: Supplementary Data (a) Dogs were removed from treatment and high-mortality occurred in groups. (b) Dogs at 0.25 mg/kg/day were not examined histologically. (c) Only 3 dogs/sex/group were used.

10. Fourteenth-Week Oral Toxicity Study in Rats Following In Utero Exposure (MSD #TT-77-043-0; No date)

Test Material: C-076 (B1a) (L-676,895); Lot OOP22

Three groups of 15 male and 15 female weanling Charles River CD strain rats were orally administered by gavage dosages of 0.1, .20 and 0.4 mg/kg/day of test material. Two identical groups served as vehicle controls and received the vehicle, sesame oil, at the same volume (5 ml/kg).

The rats in this study, obtained from five groups of F₀ female rats, had been exposed in utero to the test material and vehicle at the respective concentrations.

The rats were observed daily for toxic signs. Body weight was recorded twice a week. Ophthalmologic examinations were done on all control and high dose rats in Weeks 5, 9, and 13. Hematologic and clinical chemistry studies were done on Weeks 4, 8 and 12. Postmortem studies included necropsy of all rats and weighing of spleen, heart, kidneys, testes, liver and brain.

Microscopic examination of various tissues was done on 8 male and 8 female rats from Control I and 0.4 mg/kg/day groups plus all tissues with gross lesions.

Results:

No toxic signs or deaths were noted in any of the treatment groups.

Body weight gain of rats at 0.4 mg/kg/day was increased compared to controls.

No treatment-related ophthalmologic changes were observed. No treatment-related effects in hematology or clinical chemistry were noted.

There were no gross, organ weight, or microscopic changes that were considered treatment-related.

Conclusion: The NOEL is 0.4 mg/kg/day.

Classification: Supplementary Data (a) Inadequate pathology since 0.1 and 0.2 mg/kg/day groups were not examined. And only 8 rats/sex were examined at Control I and 0.4 mg/kg/day.