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

JUL 8 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Developmental and Reproductive Toxicity Peer Review of Avermectin

TO: George LaRocca
Insecticides/Rodenticides Branch
Registration Division (H7505C)

and

Jay Ellenberger
Reregistration Branch
Special Review and Reregistration Division (H7508W)

FROM: Roger Gardner Roger Gardner 5-20-93
Developmental/Reproductive Toxicity Peer Review Committee
Health Effects Division

and

Review Section 1, Toxicology Branch I
Health Effects Division (H7509C)

The Health Effects Peer Review Committee (PRC) for Developmental and Reproductive Toxicity met on May 20, 1992 to discuss and evaluate the weight-of-the-evidence on avermectin with particular reference to its potential for developmental and reproductive toxicity. The PRC concluded that avermectin and related compounds induced developmental toxicity in several species with the mouse being the most sensitive species. For purposes of acute dietary risk assessment, a NOEL of 0.06 mg/kg/day (based on an isomeric photodegradation product found in plants) was recommended. The most appropriate value to use for mixer/loader/applicator risk assessment is 0.05 mg/kg/day, based on maternal toxicity observed with the parent compound.

A. Individuals in Attendance

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp Penelope A. Fenner-Crisp

William L. Burnam

Karl Baetcke

Marcia Van Gemert
Bob Sonawane
Stephen Dapson
Laurence D. Chitlik
James Rowe
Hugh Pettigrew

2. Reviewers: (Non-Committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

William Dykstra
Roger Gardner
Pam Hurley
Nguyen B. Thoa
John Whalan

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Thomas F. X. Collins
Jennifer Orme Zavaleta
Jennifer Seed
Reto Engler

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Section I of Toxicology Branch I.

C. Background Information

Avermectin B_1 is a natural pesticide isolated by Merck & Co. from the soil actinomycete *Streptomyces avermitilis*. It is used against a variety of mites and insects on food crops. Avermectin B_1 is one of a class of macrocyclic sixteen-membered lactones and the active ingredient is a mixture of two compounds known as avermectin B_{1a} (~80%) and avermectin B_{1b} (~20%).

There are also closely related photodegradates found in avermectin-treated plants known as the delta 8,9 isomer, which most closely resembles avermectin B_1, and polar degradates. These degradates have been evaluated for their potential to cause developmental toxicity.

The RfD is 0.0004 mg/kg/day. This value was calculated by using a NOEL from the 2-Generation rat reproduction study (0.12 mg/kg/day) and an uncertainty factor of 300 based on the severity of effects at the LEL (Increased retinal folds in weanlings, decreased viability and lactation indices, decreased pup body weight, increased number of dead pups at birth). The RfD was verified by HED on March 30, 1989, and by EPA on April 20, 1989.

D. Studies Pertaining to Developmental Toxicity

Unless otherwise specified, the studies described below followed the standard protocol as recommended in Subdivision F test guidelines (§83-3).

1. Studies with Technical Grade Avermectin B_1

a. Developmental Toxicity Study of MK-0936 (Avermectin B_1) in Rats (Study No. TT 82-705-0; November 10, 1982; MRID 00130819)

Pregnant CRCD rats were dosed with MK-0936 (avermectin B_1; approximately 94% pure) at 0 (sesame oil vehicle control), 0.4, 0.8, and 1.6 mg/kg/day on gestation days 6 through 19.

There were no maternal deaths, abortions, or dose-related clinical signs in any group. Maternal body weights and body weight gains were comparable between all groups.

There were no dead fetuses found on day 20. As the data in Table 1 illustrate, there were no dose-related differences in the litters. The only significant differences were in the number of total resorptions and the number of live fetuses/pregnant dam in the 0.8 mg/kg/day group. These effects, which are not dose-related, are attributed to two litters.

TABLE 1

Litter Data from Rats Given Avermectin B_1 (MRID 00130819)

	Dose Levels (mg/kg/day)			
	0	0.4	0.8	1.6
No. pregnant	25	24	25	25
Corpora lutea/dam	NR	NR	NR	NR
Implantations/dam	14.3	13.8	13.2	14.1
Resorptions (total)	11	10	37	13
Live fetuses/dam	13.9	13.3	11.6	13.6
Litter weight (g)	1.88	1.88	1.73	1.89

There were several fetuses with external abnormalities, but because of the low incidence (one or two fetuses out of approximately 300 fetuses/group), they are not considered to be compound-related. The only visceral alteration of note was slightly distended ureter which was found in 3 fetuses from 3 high-dose litters. This same alteration was seen in 1 mid-dose and 2 low-dose fetuses. The low incidence and lack of clear dose response suggested that this may not be a compound-related effect. The incidence of skeletal variations was comparable between groups.

The lack of any maternal or developmental toxicity suggested that the doses selected for this study may not have been adequate.

A range-finding study (TT 82-705-1) was performed to select doses for the developmental toxicity study. Five groups of 10 mated female rats were dosed by gavage with MK-0936 at 0 (sesame oil control), 0.25, 0.5, 1.0, and 2.0 mg/kg/day on gestation days 6 through 17. One dam from the highest dosed group had tremors and lost weight (day 18 weight of 225 g compared to the group mean of 332 g). The animal was sacrificed on gestation day 18 after 12 doses. No other toxicity was observed in the other 9 dams in the high dose group.

b. Developmental Toxicity Study of MK-0936 (Avermectin B_1) in Rabbits (Study No. TT 82-706-0; November 10, 1982; MRID 00130819)

Pregnant New Zealand White rabbits were dosed with MK-0936 (avermectin B_1; approximately 94% pure) at 0 (sesame oil vehicle control), 0.5, 1.0, and 2.0 mg/kg/day on gestation days 6 through 27.

A low-dose doe died on gestation day 26 after 20 doses, a mid-dose doe died on gestation day 27 after 21 doses, and a high-dose doe was sacrificed moribund on gestation day 20 after 14 doses. Food and water consumption were reportedly decreased during the latter part of the dosing period in the low and high-dose does that died. Blood and urine were found on gestation days 26 and 27 in the waste pan beneath the mid-dose doe which died. Six control does also had decreased food and water consumption, and 2 had blood in their waste pans. The laboratory attributed these clinical signs and deaths to extra handling during a prolonged period of administration. None of the deaths in the dosed groups can be conclusively attributed to the test article.

Maternal body weight gain in the high-dose does was consistently below control values throughout the dosing period (Table 2). In contrast, body weight gain in the low-dose does was consistently above control values.

TABLE 2

Maternal Body Weight Gain (g) in Rabbits Given Avermectin B_1 (MRID 00130819)

	Dose Groups (mg/kg/day)			
	0	0.5	1.0	2.0
Body Wt. Gain (g)				
Days 6-18	+64	+59	+94	-65
Days 18-28	+8	+162	-24	-77
Days 6-28	+72	+221	+70	-142

There were abortions in each group which were not compound-related (one each in the control, low, and high dose groups and 2 in the mid-dose group). Fetal survival was comparable in all groups and there were no dose-related group differences with respect to numbers of corpora lutea, implantations or resorptions per doe.

External examination revealed one high-dose litter with two fetuses having cleft palates, and another two fetuses having omphaloceles. Three other high-dose litters had a total of 5 fetuses with clubbed forefeet. In the mid-dose group, 2 fetuses within a litter had clubbed forefeet. No malformations were found in the low-dose group. One control fetus each had omphalocele and clubbed forefeet. Considering the litter incidence of these malformations, clubbed forefeet in the high-dose fetuses is probably attributable to administration of the test article.

There were no dose-related visceral malformations or variations. Dose-related skeletal anomalies included incomplete ossification of the sternebrae and metacarpals in the high-dose group (Table 3). All other variations were of low incidence or not dose-related.

TABLE 3

Skeletal Examination in Rabbits GivenAvermectin B_1
(MRID 00130819; litter data were not reported)

	Dose levels (mg/kg/day)			
	0	0.5	1.0	2.0
Fetuses examined	97	91	100	121
Sternebral malformation	0	0	0	3
Incompletely ossified sternebra	17	17	16	42
Incompletely ossified pelvic bone	1	2	0	3
Incompletely ossified metacarpal	8	15	7	33
Incompletely ossified forefoot phalanx	19	27	12	31
Incompletely ossified talus calcaneous	2	5	2	6

There was maternal toxicity at the high dose (decreased body weight gain), and the NOEL for maternal toxicity was 1.0 mg/kg/day. Developmental toxicity observed at the high dose (2.0 mg/kg/day)

included clubbed forefeet, and incomplete ossification of the sternebrae and metacarpals. The developmental NOEL is 1.0 mg/kg/day, and the developmental LEL is 2.0 mg/kg/day.

c. MK-0936: Ten-Day Dietary Maternotoxicity Study in Mice (Study No. TT 83-705-1; April 11, 1984; Acession No.73761)

Groups of 20 mated Charles River CF1 female mice were given dose levels of 0, 0.33, 1 or 2 ppm (0, 0.1, 0.3 or 0.6 mg/kg/day) of test material in their diet from gestation day 6 through 15. Fetuses were not examined for developmental effects.

No effects were observed at 0.1 mg/kg/day. Two pregnant and one non-pregnant mice from the mid-dose group developed hunched back and marked tremors after 6-7 days of dosing and two pregnant high-dose group animals developed marked tremors after 3 days of dosing. These mice were sacrificed on the day signs were observed. Body weight or body weight gain were comparable between groups during the pre-dosing and dosing periods.

Pregnancy rates (20 controls, 20 low-, 18 mid, and 18 high-dose group animals) were comparable between groups. Other reproductive parameters were discounted because they were only estimations.

The maternal NOEL for MK-0936 administered in the diet of pregnant mice from gestation day 6 through 15 was 0.1 mg/kg/day and the LOEL was 0.3 mg/kg/day based on clinical signs of tremors necessiting early sacrifice.

2. Studies with Avermectin B_{1a}

a. Rats

i. Range-Finding Developmental Toxicity Study of Avermectin B_{1a} in Rats (Study No. TT 87-701-0; April 21, 1982; MRID 00101523)

A 4-page report described a range-finding study in which groups of 20 mated female CRCD rats were dosed orally (presumably by gavage) at 0 (sesame oil control), 0.8, 1.6, or 3.2 mg/kg/day on gestation days 6 through 15.

Three high-dose dams died after showing signs of ataxia and whole body tremors one to two days prior to death. One was comatose prior to death. Several other high-dose dams had ataxia and fine whole-body tremors. The low and mid-dose groups had no clinical signs. The fetuses from the high-dose group were evaluated and found to have no dose-related malformations.

ii. Post-Natal Study Entitled "Avermectin B_{1a} Oral Reproduction Toxicity Study in Rats" (Study No. TT #77-712-0; May 11, 1982; MRID 00096451)

Five groups of 15 female Charles River CD rats were tested with Avermectin B_{1a} in a post-natal developmental toxicity study. The following dose levels were administered orally in sesame oil in a volume of 5 ml/kg: 0.1, 0.2 and 0.4 mg/kg/day. Two control groups received 5 ml/kg sesame oil. The animals were treated for 15 days prior to cohabitation with untreated males, and dosing of the females continued throughout the mating period. The females were dosed throughout gestation and lactation until day 21 postpartum.

The following parameters were measured: parental body weights, clinical signs of toxicity and the number of metrial glands at sacrifice, and pup number/litter (including viability through day 21), weight, sex, clinical signs of toxicity, external abnormalities and litter size. The pups were also examined daily for developmental signs which included earflap opening, eye opening, incisor eruption and hair growth. They were also fixed for subsequent visceral examination by dissection. Dead pups were stained and examined for skeletal malformations.

There were no treatment-related effects on the dams throughout the dosing period. There were no effects on the mating, reproductive parameters, average length of gestation or postimplantation survival rate at any dose level.

There were also no treatment-related effects on pup mortality during lactation with the exception of lactation day 1 (see Table 4). Clinical signs of toxicity were observed in pups in the 0.2 and 0.4 mg/kg/day groups which included spastic movements of the limbs and muscular tremors of the entire body (high dose group), and pup weights in the highest dosed group were significantly decreased in comparison with control values during lactation (see Table 4). External, visceral and skeletal examinations of the dead pups revealed no evidence of treatment-related effects. Post-natal changes noted in the report are summarized in Table 5 below.

The maternal NOEL was > 0.4 mg/kg/day (highest dose tested), and the developmental NOEL was 0.1 mg/kg/day. The LEL for developmental toxicity was 0.2 mg/kg/day based on the increased incidence of spastic movements of the limbs of pups during lactation, decreased pup body weights at lactation days 7 and 21, and decreased litter size at day 1 of lactation.

TABLE 4

Selected Pup Mortality and Body Weight Data from a Postnatal Study with Avermectin B_{1a} in Rats (MRID 00096451)

	Dose Levels (mg/kg/day)				
	0	0	0.1	0.2	0.4
# Pregnant ♀	11	12	13	13	12
Day 1 Postpartum					
# Live pups (per dam)	141 (12.8)	146 (12.2)	172 (13.2)	175 (13.5)	141 (11.8)**
# Dead pups (per dam)	2 (0.2)	2 (0.2)	1 (0.1)	4 (0.3)	7 (0.6)
Day 7 Postpartum					
Ave. pup wt. (g/litter)	12.9	12.9	13.8	12.9	11.2*
Day 14 Postpartum					
Ave. pup wt. (g/litter)	27.8	27.8	29.4	28.2	23.3
Day 21 Postpartum					
Ave. pup wt. (g/litter)	45.0	45.0	47.3	47.0	39.4*

* Statistically significant from pooled controls $p \leq 0.05$.
** Statistically significant from pooled controls based on rankit adjusted treatment means $p \leq 0.05$.
1 control dam had lost entire litter by day 7.

TABLE 5

Mean Age (Days[1]) For Occurrence of Selected Developmental Observations in Pups Exposed to Avermectin B_{1a} (MRID 00096451)

	Dose Levels (mg/kg/day)				
Observation	0	0	0.1	0.2	0.4
Ear Opening	5.0	5.2	5.2	5.4	6.1*
Incisor Eruption	12.5	13.0	12.4	12.0*	12.1*
Hair Growth	12.5	13.1	12.1	12.5	13.5*
Eye Opening	16.2	16.6	16.3	16.4	17.4*

* Significantly different ($p \leq 0.05$) from pooled controls.
1 Age at which all pups in a litter displayed the developmental sign.

iii. Post-Natal Study Entitled "Avermectin B_{1a} Oral Reproduction Toxicity Study in Rats" (Study No. TT #77-706-0; May 11, 1982; MRID No. 00096450).

In a second post-natal developmental toxicity study on Avermectin B_{1a}, the test chemical was administered orally at higher dose levels (0.5, 1.0 and 2.0 mg/kg/day) to groups of 12 female albino CD rats. Other aspects of the study protocol were the same as those described in the previous section.

In the highest dose group, two females died and a third was sacrificed after 9 to 15 doses. Clinical signs of toxicity included whole body tremors, ataxia, ptyalism and ocular and/or nasal discharges. Prior to reduction of the high dose to 1.5 mg/kg/day, one dam displayed tremors. There were no mortalities or clinical signs of toxicity in any of the other dams in this dose group or in any other dose groups. Body weights, body weight gains, breeding, reproductive performance, abortions and postimplantation survival rate were not affected in any of the dose groups (only including surviving dams in high dose group).

Effects on pup observations are summarized in Table 6 below.

The NOEL for maternal toxicity was 1.0 mg/kg/day and the LEL was 2.0/1.5 mg/kg/day based on whole body tremors, ataxia, ptyalis, occular/nasal discharges and mortality. The developmental toxicity NOEL was < 0.5 mg/kg/day (lowest dose tested) based on decreased pup weight and postnatal survival.

Table 6

Selected Litter Data from a Postnatal Study with Avermectin B_{1a} in Rats (MRID No. 00096450)

	Dose levels (mg/kg/day				
Observation	0	0	0.5	1.0	1.5
# Pregnant ♀	10	6	11	10	8
Day 1 Postpartum					
Total # pups	123	84	135	122	84
# Implantation sites (per dam)	13.6	15.2	13.6	12.7	12.4
# Live pups (per dam)	120 (12)	84 (14)	134 (12.2)	117 (11.7)	79 (9.9)
# Dead pups (per dam)	3 (0.3)	0 (0.0)	1 (0.09)	5 (0.5)	5 (0.63)
Ave. pup wt. (g/litter)	6.39	6.39	6.50	6.41	5.96*

Table 6

Selected Litter Data from a Postnatal Study with Avermectin B_{1a} in Rats (MRID No. 00096450)

Observation	Dose levels (mg/kg/day				
	0	0	0.5	1.0	1.5
Day 7 Postpartum					
# Live pups after reduction (per dam)	74 (7.4)	47 (7.8)	79 (7.2)	38 (3.8)	21 (2.6)
Cumulative total # dead pups	5	1	4	41	46
# Dead pups days 2-7 (per dam)	2 (0.2)	1 (0.2)	3 (0.3)	36 (3.6)	41 (5.1)
Ave. pup wt. (g/litter)	12.60	12.60	9.79*	6.86*	4.91*
Day 14 Postpartum					
# Live pups (per dam)	74 (7.4)	47 (7.8)	64 (5.8)	11 (1.6)	0 (0.0)
Cumulative total # dead pups	5	1	19	68	67
# Dead pups days 8-14 (per dam)	0 (0.0)	0 (0.0)	15 (1.4)	27 (3.9)	21 (4.2)
Ave. pup wt. (g/litter)	26.75	26.75	15.82*	12.06*	N.A.[1]
Day 21 Postpartum					
# Live pups (per dam)	74 (7.4)	47 (7.8)	62 (5.6)	10 (3.3)	0 (0.0)
Cumulative total # dead pups	5	1	21	69	67
# Dead pups days 15-21 (per dam)	0 (0.0)	0 (0.0)	2 (0.2)	1 (0.3)	N.A.[1]
Ave. pup wt. (g/litter)	44.27	44.27	27.06*	18.18*	N/A[1]

* Statistically significant, $p \leq 0.05$.
[1] Not applicable - no surviving pups in group by day 10.

b. Range-Finding Developmental Toxicity Study of Avermectin B_{1a} in Rabbits (Study No. TT 76-724-2 and 77-702-0/-1; April 21, 1982; MRID 00101523)

A 6-page report of a range-finding study indicated that groups of 4 artificially inseminated female rabbits were dosed orally (presumably by gavage) at 0 (sesame oil control), 0.25, 0.5, 1.0, 2.0, and 4.0 mg/kg/day on gestation days 7 through 16.

Three high-dose does died after 4-7 doses. Clinical signs seen in the 2.0 and 4.0 mg/kg/day groups included stupor,

mydriasis, and weight loss. In addition, the does which died had intermittent whole body muscular tremors and coma.

A longer report in the same volume described a developmental toxicity study using groups of 25 artificially inseminated rabbits dosed at 0 (two sesame oil control groups), 0.25, 0.5, and 1.0 mg/kg/day. This study was stopped and repeated due to an unusually low fertility rate and high incidence of intubation accidents resulting in an insufficent number of litters.

In the repeat study, there were no treatment-related mortalities or clinical signs. There was reportedly a slight statistically significant decrease in average maternal body weight gain in the 1.0 mg/kg/day group. External, visceral, and skeletal examinations of all fetuses did not reveal any evidence of developmental toxicity. The high-dose fetuses had a slight, but statistically significant decrease in average live fetal weight per litter.

The study author stated, "The results of this study should be considered as preliminary and will be confirmed in repeat oral range-finding and teratogenic studies in rabbits. The protocol for these studies will conform to current EPA guidelines." It is not clear which studies subsequently submitted by the registrant are intended to confirm the preliminary results described here.

c. Mice

i. Ten-Day Oral Toxicity Study (Range-Finding) in Pregnant Mice (Study No. TT 77-717-1; undated; MRID No. 00096446)

Groups of 20 mated CF1 mice were administered 10 ml/kg vehicle containing 0, 0.025, 0.050, 0.075, and 0.10 mg/kg/day of test material (C-076 B_{1a}) by oral gavage from gestation day 6 to 15.

One mouse treated with 0.1 mg/kg/day was found dead after 3 dosings. One mouse treated with 0.075 mg/kg/day was sacrificed in a comatose state after 4 dosings. Death/sacrifice was preceeded by tremors and coma. Of the surviving mice, only 2 in the high dose group also showed tremors. No other clinical signs were observed.

Average maternal weight gains were comparable between groups. Pregnancy rate was comparable between groups but was very low [12 controls (60%), 12 treated with 0.025 mg/kg/day (60%), 14 treated with 0.05 mg/kg/day (70%), 12 treated with 0.075 mg/kg/day (60%), and 10 treated with 0.1 mg/kg/day (50%)].

The maternal NOEL was 0.05 mg/kg/day, and the LOEL was 0.075 mg/kg/day based on one death. The highest dose tested (0.1 mg/kg/day) caused one death after 3 dosings, but this dose was

chosen as the lowest dose in the definitive study (TT 77-705-0; see below).

ii. Oral Teratogenic Evaluation in Mice (Study No. TT 77-705-0; undated; MRID No. 00096446) and Addendum, Supplemental Information on Skeletal Data (MRID No. 00164014)

Groups of 20 mated CF1 mice were administered 10 ml/kg sesame oil containing 0 (control 1), 0 (control 2), 0.1, 0.2, 0.4, and 0.8 mg/kg/day of test material by oral gavage from day 6 through 15 of gestation. There was no mention that clinical signs were noted in the study.

Mortality was observed in every dose group except at the 0.2 mg/kg/day leve. Respective death rates were 1, 3, and 2 in the groups treated with 0.1 (after 8 dosings), 0.4 (after 2 dosings), and 0.8 mg/kg/day (after one dosing). Death was preceeded generally by tremors, then coma. Body weight of all surviving mice were unaffected by the test material. There was no mention of any clinical signs among the survivors.

The only effect observed was a slight decrease in average fetal weight/dam in the lowest dosed group (16 litters) which only attained statistical significance ($p < 0.05$) when the data from both control groups (35 litters) were combined (control = 0.89 g; lowest dosed group = 0.84 g). This observation may be incidental since it was not dose-related and may be biased by the unmatched numbers of litters used for the statistical evaluation.

There was an increase in the incidence of cleft palate in the 0.4 and 0.8 mg/kg/day dose groups (4 and 5 fetuses [2.4% and 2.5%], respectively). Although the litter incidences in these 2 groups (2/14 and 2/17 litters, respectively) were not dose-related and were not statistically significantly different from concurrent control incidence compared with no incidences in the two control groups ($p > 0.05$; Fisher Exact pair-wise comparison); they were within historical ranges on a fetal basis (0.28-3%) or a litter basis (3-14.3%). Historical control data included 24,628 fetuses from 2076 litters from an unreported number of studies.

The incidence of other external abnormalities was not significantly increased in treated groups, and fetal visceral observations were comparable for all test groups.

Skeletal malformations/variations were comparable between groups except for the incidence of 14th lumbar rib (0/184, 8/234, 7/195, 8/242, 6/165, and 13/199 in the two control groups, 0.1, 0.2, 0.4, and 0.8 mg/kg/day dose groups, respectively). No litter data were provided for this alteration.

A maternal NOEL was not established because the lowest dose tested (0.1 mg/kg/day) caused one death. Mortality was not dose-

related, but the higher doses appeared to cause earlier deaths. The developmental NOEL was 0.2 mg/kg/day and the LOEL was 0.4 mg/kg/day based on increased incidence of cleft palate.

iii. Oral Range-finding Studies (Study Nos. TT-76-723-1/2; undated; MRID No. 96447)

Groups of 5 mated CF1 mice were administered doses of 0.1, 0.25, or 0.5 mg/kg/day test material in study No. TT-76-723-1 and 1, 2, 4, 6, or 8 mg/kg/day test material in study No. TT-76-723-2, by oral gavage, from gestation day 6 through 15. Mortality was observed, but the death rates were inconsistent (1 death at ≤ 1 mg/kg/day; no death at 4 and 6 mg/kg/day; 3 deaths at 8 mg/kg/day). Death was preceeded by tremors and ensuing coma. Because the inconsistent mortality may be due to an inconsistent absorption of the test material (suspended in methyl cellulose for these studies) the vehicle was changed to sesame oil in 2 replicate definitive studies.

iv. Oral Teratogenic Studies (Replicates Study Nos. TT 76-723-0/-3; undated; MRID No. 00096447)

In the two replicated studies, groups of 10 (study No. TT 76-723-0) or 15 (study No. TT 76-723-3) mated CF1 mice were administered 10 ml/kg sesame oil containing 0, 0.1, 0.2, 0.4, or 0.8 mg/kg/day of test material by oral gavage from day 6 through 15 of gestation. The data from both studies were combined to achieve a meaningful number of litters for evaluation. Fetal data were not statistically analyzed.

Mortality was observed in every dose group, and the incidences were dose-related. Respective death rates were 1, 3, 6, and 8 for the groups treated with 0.1, 0.2, 0.4, and 0.8 mg/kg/day. The mouse from the lowest dosed goup died on gestation day 18 and 7 of the 8 deaths occurred in the highest dosed group after one dosing. Death was preceeded generally by tremors then coma. Body weight gain of all surviving mice were unaffected by the test material. Reproductive parameters were also comparable between groups of survivors.

No developmental effects were observed at doses ≤ 0.2 mg/kg/day. In the 0.4 and 0.8 mg/kg/day dose groups, there was a dose-related increase in fetal incidence (5 and 10 fetuses [2% and 5%, respectively]) and litter incidence (2/19 and 4/16 litters, respectively) of cleft palate. Although the litter incidences in the two highest dosed groups were not statistically significantly different from concurrent control incidence (0 fetuses affected), the highest dosed group incidence (25%) was outside historical ranges on a litter basis (3-14.3%). Historical control data included 24,628 fetuses from 2076 litters from an unreported number of studies. Affected fetuses had normal size.

Fetal visceral and skeletal malformations/variations observations were comparable in all test groups.

A maternal NOEL was not established because the lowest dose (0.1 mg/kg/day) caused one death. Mortality was dose-related. The developmental NOEL was 0.2 mg/kg/day and the LOEL was 0.4 mg/kg/day, based on an increase of fetal/litter incidences of cleft palate. Both replicate studies were classified Core Supplementary, because "fetuses at 0.1 and 0.2 mg/kg/day were not examined."

3. Avermectin B_{1b}: II. Oral Maternotoxicity Study in Mice (Study No. TT 84-721-0; March 4, 1985; MRID No. 00164020)

Avermectin B_{1b} was solubilized in sesame oil and given by oral gavage to groups of 12 mated Charles River CF1 female mice at dose levels of 0, 0.025, 0.05, 0.075, or 0.1 mg/kg/day from gestation day 6 through 15.

Two deaths were observed in the 0.075 mg/kg/day dose group. Both appeared to be treatment-related because they were preceeded by tremors (prior to dosing + 3-5 hrs post-dose on day 11 and 12), and similar responses were observed in mice treated with avermectin B_1 and B_{1a}. No other adverse effects were observed in adult test animals, and reproductive parameters were statistically comparable between groups.

There was one incidence of exencephaly each in the 0.025 and 0.1 mg/kg/day dose groups, and one incidence of cleft palate each in the 0.05 and 0.075 mg/kg/day dose groups. The incidence of cleft palate is not dose-related (1/108 or 0.93%, 1/111 or 0.9% and 0 % of the fetuses in the 0.05, 0.075 and 0.1 mg/kg/day dose groups, respectively). A comparison of the incidence of cleft palate with historical control data described above indicates that fetal and litter incidences (1/9 or 11.1% and 1/12 or 8.3% in the 0.05 and 0.075 mg/kg/day dose groups, respectively) are within historical ranges (0.28% to 3% for fetal incidence and 3 to 14% for litter incidence). Historical data for the incidence of exencephaly in mice were unavailable for comparison with results from this study.

The NOEL for maternal Toxicity was 0.05 mg/kg/day. The LOEL was 0.075 mg/kg/day based on 2 mortalities (16%). A NOEL for developmental effects could not be established because the fetuses were not examined for soft tissues or skeletal alterations.

4. Studies with Avermectin B_1 delta 8,9-Isomer

a. Rats

i. Developmental Toxicity Study of Delta 8,9-Isomer, Avermectin B_1 in Rats (Study No. TT 87-715-0; June 7, 1988; MRID 407134-03)

Four groups of pregnant Sprague-Dawley [Crl:CD(SD) BR] rats were dosed by gavage with the delta 8,9-isomer of avermectin B_1 at 0 (sesame oil vehicle control), 0.25, 0.5, and 1.0 mg/kg/day on gestation days 6 through 17.

There were no maternal deaths, abortions, or dose-related clinical signs in any group. Maternal body weights, body weight gains, and food consumption were comparable between all groups.

There were no dead fetuses found on day 20, and there were no dose-related differences in litter observations.

There were also no dose-related external, visceral, or skeletal defects observed in fetuses from treated dams.

ii. Post-Natal Study Entitled "Single Generation Reproduction Study of the Delta 8,9-Isomer of Avermectin B_1 in Rats (Study No. TT 87-716-0; June 7, 1988; MRID 407134-04)

Female Sprague-Dawley [Crl:CD(SD) BR] rats (10 weeks old) were mated with untreated males. Eighty females with the presence of sperm in a vaginal lavage were assigned to four groups of 20 dams each. They were dosed with the delta 8,9-isomer of avermectin B_1 (91.6% pure) at 0 (vehicle control), 0.06, 0.12, and 0.40 mg/kg/day 15 days prior to cohabitation, throughout gestation, and through lactation day 20. The test article was formulated in sesame oil and administered by gavage at 5 ml/kg. The rats were observed for clinical signs at least once daily. Maternal body weights and food consumption were recorded at regular intervals.

The length of gestation was recorded. The dams delivered naturally. The F_1 pups were examined externally on lactation day 0 (day of birth). Ten pups from each litter were sexed, randomly selected and tattooed. On lactation day 3, each litter was culled to 8 pups, with the unneeded pups being sacrificed without examination. The pups were weighted on lactation days 0, 7, 14, and 21. They were observed daily for clinical signs until lactation day 21 when they were sacrificed. Necropsy was limited to removal of the eyes, which were histomorphologically examined.

The dams were sacrificed on lactation days 21-23 and necropsied. Their metrial glands were counted.

There was no evidence of toxic effect in the dams at any time in the study. Maternal body weights, body weight gains, food consumption, and reproductive parameters were comparable between all groups.

There was no evidence of toxic effect in the F_1 pups.

b. Mice

i. 8,9 Isomer of Avermectin B_1 (L-652,280-00N) I. Oral Maternotoxicity Study in Mice (Study No. TT 84-722-0; January 8, 1986; MRID No. 00164011)

Groups of 8-13 mated Charles River CF1 mice were administered 0, 1.5, 3.0, 6.25, 12.5, 25, or 50 mg/kg/day of test material (in sesame oil; dose-volume = 10 ml/kg) by oral gavage from day 6 through 15 of gestation.

Mortality was observed at all the doses used (2-3 deaths at ≥3 mg/kg/day after 1 dose; 1 death at 1.5 mg/kg/day after 2 doses). Some of the deaths were preceeded by coma. All groups except the control and lowest dosed group were terminated from day 6 to 8. No maternal effects were observed in the surviving mice.

The pregnancy rate and the number of implantations, resorptions and live fetuses (total or per litter) of the control and the 1.5 mg/kg/day group were comparable.

Cleft palate was not observed in the control groups, but at the 1.5 mg/kg/day dose level 24 of 43 fetuses (56%) were affected in 4 of 7 litters (57%). These values were statistically significant and outside historical control ranges (from studies involving 24,628 fetuses from 2079 litters (ref: study No. TT-84-722-1): Fetal = 0.28-3%; Litter = 3.0-14.3%).

ii. 8,9 Isomer of Avermectin B_1 (L-652,280-00N) II. Oral Maternotoxicity Study in Mice (Study No. TT 84-722-1; February 29, 1985; MRID No. 00164011)

Groups of 25 mated Charles River CF1 mice were administered doses of 0, 0.05, 0.1, 0.5 or 1 mg/kg/day of test material (in sesame oil) by oral gavage from gestation day 6 through 15.

Mortality was observed at 0.5 and 1 mg/kg/day. One mouse given the 1 mg/kg/day dose was found dead on day 10, after 4 dosings; this mouse was lethargic after one dosing and remained so until death. One mouse treated with 0.5 mg/kg/day was sacrificed "in poor condition" on day 11, after 6 dosings. This mouse had tremor prior to dosing and 1-5 hours post-dose on day 10 and 11. No adverse changes were observed in any of the surviving mice from every group.

Statistically significant decreases in the number of implantations/dam (12.5 and 9.8 for the control and highest dosed groups, respectively) and in the number of live fetuses/dam (11.3, 8.7, and 8.3 at 0, 0.05 and 1 mg/kg/day doses, respectively) were observed. All other reproductive parameters were comparable between groups.

There was an increase in fetal and litter incidences of cleft palate and exencephaly (See Table 7 below).

Table 7

Selected Fetal External Alterations in Mice Given the 8,9 Isomer of Avermectin B_1 (MRID 00164011)

	Dose level (mg/kg/day)				
Observation	0	0.05	0.1	0.5	1.0
No. examined					
Fetuses	136	104	115	90	91
Litters	12	12	11	9	11
With cleft palate					
Fetuses	0	0	13	1	7
(%)	0	0	(11.3)*	(1.1)	(7.7)*
Litters	0	0	2	1	4
(%)	0	0	(18.2)*	(11.1)	(36.4)*
With exencephaly					
Fetuses	1	0	2	4	2
(%)	(0.7)	0	(1.73)	(4.4)*	(2.2)*
Litters	1	0	1	3	1
(%)	(8.3)	0	(9.0)	(33.3)*	(9.0)

Historical Control Data

	Range of Fetal Incidences	Range of Litter Incidence
Cleft Palate	0.28-3%	3.0-14.3%
Exencephaly	0.26-1.6%	2.7-14.3%

* Outside historical control ranges (based on 24,628 fetuses from 2079 litters.

iii. 8,9 Isomer of Avermectin B_1 (L-652,280-00N) III. Oral Teratology Study in Mice (Study No. TT 85-710-0; June 4, 1985; MRID No. 00164011)

Groups of 25 mated Charles River CF1 mice were administered 0, 0.015, 0.03, or 0.06 mg/kg/day of test material (in sesame oil) by oral gavage, from gestation day 6 through 15.

The dose range used (0.015-0.06 mg/kg/day) was not sufficiently high to cause maternal, reproductive, or developmental effects. The maternal NOEL was ≥ 0.06 mg/kg/day (HDT), and the developmental NOEL ≥ 0.06 mg/kg/day.

iv. 8,9 Isomer of Avermectin B_1 (L-652,280-00N) IV. Oral Teratology Study in Mice (Study No. TT 85-710-1; September 16, 1985; MRID No. 00164011)

Groups of pregnant Charles River CF1 mice were administered doses of 0, 0.015, 0.03, 0.1, and 0.5 mg/kg/day of test material (in sesame oil) by oral gavage from day 6 through 15.

One mouse treated with the 0.5 mg/kg/day dose was sacrificed after 6 dosings, on day 12. This mouse lost 6.3 g between day 9-11, showed lethargy, and had chromodacryorrhea from day 9 til sacrifice. There were no other adverse maternal effects observed; body weight gain, feed consumption, and necropsy observations were comparable between groups.

There was a statistically significant increase in the incidences of dead/resorbed fetuses per dam in the 0.03 mg/kg/day dose group (16.3% compared with 9% in the control group). This observation was not a dose-related response. All other reproductive parameters were comparable between groups.

There was a statistically significant increase in the fetal incidences of cleft palate in the 0.1 and 0.5 mg/kg/day groups (0, 2.1 and 10.3% in the 0, 0.1 and 0.5 mg/kg/day dosed groups, respectively). The litter incidence of the 0.5 mg/kg/day group (0/23 in controls compared with 5/23 in the highest dosed group) was also statistically significantly higher than concurrent control incidence (Fisher exact test; $p < 0.02$), and there was a positive trend (Cochran Armitage test; $p < 0.002$; 0/23, 1/24, 1/23, 1/24 and 5/23 affected litters in the control, 0.015, 0.03, 0.1 and 0.5 mg/kg/day groups, respectively). The fetal and litter incidences of cleft palate in the highest dosed group were outside the historical range (>3% and >14.3% for fetal and litter incidences, respectively).

Fetal visceral and skeletal effects were only single incidences or occurred without a dose-response relationship.

The dose-range used in this study (0.015, 0.03, 0.1, and 0.5 mg/kg/day) was adequate although one HDT mouse had to be sacrificed in poor condition after 6 dosings. The maternal NOEL was 0.1 mg/kg/day (second highest DT). The maternal LOEL was 0.5 mg/kg/day (HDT), based on one mortality. The developmental NOEL was 0.03 mg/kg/day in this study. The developmental LOEL was 0.1 mg/kg/day, a non-maternally toxic dose, based on increase incidence of cleft palate.

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

The test material, L-930,406 (lot 930,406-000N001), was a mixture of the polar degradates of MK-0936. It was suspended in 0.5% aqueous methyl cellulose vehicle (10 ml/kg) for administration by oral gavage.

Groups of 25 mated Charles River CF1 mice were given doses of 0, 0.25, 0.5, or 1.0 mg/kg/day of test material from gestation day 6 through 15.

No maternal toxicity or mortality was observed at any dose. Body weight gain and feed consumption were comparable between groups. Necropsy observations were also comparable between groups. Reproductive parameters were also comparable between groups.

Cleft palate and exencephaly were observed, but both the litter and fetal incidences were comparable between groups and were below historical control incidences. Other visceral and skeletal effects were single incidences or were comparable between groups.

L-930,406, administered by oral gavage to pregnant CF1 mice, at the doses of 0, 0.25, 0.5, and 1.0 mg/kg/day, did not produce any maternal or developmental effects. Both the maternal and developmental NOEL's were > 1 mg/kg/day (highest dose tested). A higher dose-range could have been used since the test material has low oral acute toxicity in CF1 mice (oral LD_{50} > 5000 mg/kg; limit test; pp.12, MRDR Position document on the toxicology of Abamectin and proposed Acceptable Daily Intake; 09/12/86; Accession No. 265564).

E. Reproduction Studies - Reproductive Effects of MK-0936 Administered Orally by Gavage to Crl:COBS™CD™(SD)BR Rats for Two Generations (Study No. TT # 82-9010, May 10, 1984; Accession No. 265576)

Avermectin B_1 was tested by gavage in a 2-generation reproduction study in male and female Crl:COBS™CD™(SD)BR rats at the following dose levels in sesame oil: 0, 0.05, 0.12 and 0.40 mg/kg/day. Thirty rats/sex/dose group were tested. The F_0 adults were mated twice, producing 2 litters, F_{1a} and F_{1b}. Selected weanlings from the F_{1b} litters were mated twice to produce the F_{2a} and F_{2b} litters. The F_0 parental animals were given the test material beginning at age 39 days for 68 days prior to the first cohabitation. The F_{1b} weanlings selected to produce the second generation were given the test material starting at 21 days and continuing for 70 days post weaning prior to the first cohabitation.

Selected weanlings (from F_{1b} and F_{2b} generations) and selected adults were necropsied and the specific tissues were examined microscopically. In addition, selected weanlings from the F_{2b} generation (10 males and 10 females/dose group) were taken for skeletal examinations.

The following observations were recorded: parental clinical signs of toxicity and body weights; reproductive parameters; mating confirmations; delivery and lactation observations; pup viability, sex and body weights; and gross external, visceral and skeletal examinations on pups which died during the lactation period.

Reproductive effects observed in the stable were not considered to be biologically significant because they did not occur consistently in matings from a given generation, and they were not observed in both generations of the study. Mating and fertility indexes were comparable for all groups during the study.

High dose F_{1b} male and female rats selected for the second generation had smaller average body weights than the controls; the high dose F_{1b} male rats continued to have decreased body weights up to day 28 ($p < 0.01$). After that time, the body weights were either comparable or greater than controls.

During the first 13 days of lactation, high dose dams tended to have a lower body weight gain when compared to controls. This effect was consistently statistically significant with the F_0 dams producing the F_{1a} generation. With the other generations, the effect was observed but tended not to be statistically significant.

The test substance had no effect on gestation index, but it reduced the lactation and viability indexes of pups as well as

reducing pup body weight at the 0.40 mg/kg/day dosed group. These results are summarized as follows:

Dose (mg/kg/day):	0	0.05	0.12	0.40
Data for F_0 to F_{1a} Generation				
Viability Indices				
Days 1-4 (%)	94.2	97.0	97.6	96.0
Days 4-7 (%)	100.0	100.0	99.6	79.3**
Days 4-14 (%)	99.5	100.0	99.6	53.2**
Lactation Index (%)	99.5	100.0	99.2	52.7**
Pup weight (g/litter)				
Day 7	12.8	13.8	13.8	10.4**
Day 14	26.4	27.5	28.2	17.8**
Day 21	41.0	42.6	42.9	27.6**
Selected Litter Data for F_0 to F_{1b} Generation				
Viability Indices				
Days 1-4 (%)	99.6	99.2	96.7	100.0
Days 4-7 (%)	99.5	100.0	99.6	94.3
Days 4-14 (%)	98.0	98.5	99.2	62.1**
Lactation Index (%)	98.0	98.5	99.2	60.0**
Pup weight (g/litter)				
Day 7	13.8	14.3	14.5	11.3**
Day 14	28.9	29.2	29.6	19.6**
Day 21	45.1	45.1	46.1	31.4**
Selected Litter Data for F_{1b} to F_{2a} Generation				
Viability Indices				
Days 1-4 (%)	98.2	98.7	99.2	98.6
Days 4-7 (%)	100.0	100.0	100.0	98.9
Days 4-14 (%)	100.0	100.0	100.0	94.4**
Lactation Index (%)	100.0	100.0	99.5	93.9**
Pup weight (g/litter)				
Day 7	12.9	13.3	13.3	12.0*
Day 14	26.7	27.1	27.1	20.6**
Day 21	42.5	42.9	42.7	32.9**

Dose (mg/kg/day):	0	0.05	0.12	0.40
Selected Litter Data for F_{1b} to F_{2b} Generation				
Viability Indices				
Days 1-4 (%)	95.8	99.2	99.0	97.5
Days 4-7 (%)	100.0	100.0	99.4	98.6
Days 4-14 (%)	100.0	100.0	99.4	93.5**
Lactation Index (%)	100.0	99.1	99.4	92.8**
Pup weight (g/litter)				
Day 7	13.4	13.7	14.2	11.7*
Day 14	28.5	27.3	27.3	20.7**
Day 21	45.9	42.5	42.9	33.2**
Retinal rosettes in F_{2b} weanlings	2/57 (♂) 4/51 (♀)	0/26 (♂) 2/34 (♀)	5/88 (♂) 0/85 (♀)	6/63 (♂) 12/66 (♀)

*= statistically significant $p \leq 0.05$; **= statistically significant $p \leq 0.01$.

The NOEL's for systemic and reproductive toxicity were ≥ 0.40 mg/kg/day, and the NOEL for developmental toxicity was 0.12 mg/kg/day. The LEL was 0.40 mg/kg/day and was based on decreased pup body weight and viability during lactation as well as an increased incidence of retinal rosettes in F_{2b} weanlings.

F. Other Aspects of Toxicity

1. Subchronic and Chronic Toxicity Data

Study	Study No.	Results	Status
MK-0936 (technical avermectin B_1)			
1-Year Feeding - Dog	82-104-0	NOEL = 0.25 mg/kg/day LEL = 0.50 mg/kg/day (high incidence of mydriasis in males and females) Mortalities at 1.0 mg/kg/day	Guideline
2-Year Feeding - Rat	82-099-0	Oncogenic potential: negative up to 2.0 mg/kg/day (HDT) NOEL = 1.5 mg/kg/day LEL = 2.0 mg/kg/day (treatment-induced tumors in both sexes) Body weight increases in males and females at all doses.	Minimum

Study	Study No.	Results	Status
21-Month Feeding/ Carcinogenicity - Rat	83-002-0,-1,-2,-3	Oncogenic potential: negative up to 8 mg/kg/day (HDT) NOEL = 4.0 mg/kg/day LEL = 8.0 (increased mortality in males, tremors and body weight loss in females, dermatitis in males and extramedullary hematopoiesis in spleen of males)	Minimum
		Avermectin B_{1a} (C-076)	
18-Week Gavage - Dog	76-073-0	NOEL = 0.25 mg/kg/day LEL = 0.5 mg/kg/day (body tremors, one death, pathology of liver, decreased body weight)	Minimum
98-Day Gavage - Rat	77-043-0	NOEL >0.4 mg/kg/day (HDT) NOTE: Rats used in this study had been previously exposed to the test material in utero.	Minimum

Reference: *One-Liners*, dated June 4, 1990

2. Mutagenicity Studies

Study	Study No.	Results	Status
		MK-0936 (technical avermectin B_1)	
Ames Assay	82-8051	Negative	Acceptable
Ames Assay	85-8005	Negative	Acceptable
Ames Assay	85-8013	Negative with S-9 activation; Mutagenicity without S-9 activation could not be evaluated in the absence of positive control.	Unceptable
Chromosome aberration - CHO cells	85-8631	Negative	Acceptable
Cytogenetic - mouse bone marrow	83-900-6	Negative	Acceptable

Study	Study No.	Results	Status
		MK-0936 (technical avermectin B_1)	
Genotoxic - rat hepatocytes	82-8520, 23, 25	Avermectin 0.3 and 0.6 mM) caused an induction of single strand DNA breaks in rat hepatocytes in vitro. No effect was observed when the assay was carried out on hepatocytes from rats dosed *in vivo* at the LD_{20} dose level (10.6 mg/kg).	Acceptable
Point mutation - V-79 mammalian cells	82-8506, 10, 19	Positive response in the presence of S-9.	Acceptable
		Avermectin B_{1a} (C-076)	
Ames Assay	76-8052	Negative	Acceptable
		Delta-8,9-isomer of avermectin B_1	
Ames Assay	87-8046	Negative	Acceptable
		Polar degradates of avermectin B_1	
Ames Assay	87-8047	Negative	Acceptable

Reference: *One-Liners*, dated June 4, 1990

3. Metabolism/pharmacokinetics/physico-chemical data:

Study	Study No.	Results	Status
		MK-0936 (technical avermectin B_1)	
Metabolism - rat	ARM-1	68.7-81.6% of label is excreted in the feces by day 7. $T_{\frac{1}{2}}$ = 1.2 days	Supplementary
Metabolism - rat	N/A	No bioaccumulation; two metabolites found: 24-hydroxy methyl Avermectin B_{1a}, and 3" desmethyl Avermectin B_{1a}	Minimum
Dermal absorption - monkey	PS#1	The maximum dermal absorption of Avermectin B_{1a} is 1% of applied dose.	Supplementary

Reference: *One-Liners*, dated June 4, 1990

G. Structure-Activity Relationships

1. General Discussion

Avermectin B_1 is a member of a family of substances known as avermectins (see Table 9). This family of chemicals interferes with interneuron and neuromuscular transmission in nematodes and arthropods, and the mechanism of action is believed to involve gamma amino butyric acid (GABA) receptors. The Series 1 compounds, ivermectin and avermectin B_1, are most active and are used in vetenary medicine (ivamectin) and as pesticides. There are developmental toxicity studies on ivermectin that show effects similar to avermectin B_1 (see next Section).

Table 9: AVERMECTIN FAMILY
(16-membered macrocyclic lactones)

A-COMPOUNDS (5-methoxy substituent)				B-COMPOUNDS (5-hydroxy substituent)			
Avermectin A_1 (22,23-double bond obtained by dehydration of the axial 23-hydroxy group in Avermectin A_2)		Avermectin A_2		Avermectin B_1 - Abamectin * (22,23-double bond obtained by dehydration of the axial 23-hydroxy group in Avermectin B_2)		Avermectin B_2	
A_{1a} (>80%) secondary butyl sidechain at C-25)	A_{1b} (<20%) isopropyl substituent at C-25)	A_{2a} (>80%) secondary butyl sidechain at C-25)	A_{2b} (<20%) isopropyl substituent at C-25)	B_{1a} (>80%) secondary butyl sidechain at C-25)	B_{1b} (<20%) isopropyl substituent at C-25)	B_{2a} (80%) secondary butyl sidechain at C-25)	B_{2b} (<20%) isopropyl substituent at C-25)
				Photodegradates: † Δ-8,9-isomer of avermectin B_{1a} & Polar degradates			

* Abamectin is the generic name for Avermectin B_1 (MK-0936). It is used as the starting material for the production of the semisynthetic 22,23-dihydro analog generically known as Ivermectin. Ivermectin contains at least 80% 22,23-dihydroavermectin B_{1a}, and less than 20% 22,23-dihydroavermectin B_{1b}. Ivermectin is the generic name for Heartgard 30®, Cardomec®, Eqvalan®, Ivomec®, Zimecterin®, and Mectizan®

† The Δ-8,9 isomer is formed when avermectin B_{1a} is exposed to light. It comprises 1-4% of the total avermectin B_1 residue found on citrus after 1-2 weeks. The polar degradates are not structurally similar to abamectin.

Reference: William C. Campbell, ed. *Ivermectin and Abamectin*. Springer-Verlag, New York. 1990.

2. Studies with Ivermectin

Test compound	Species	Results
22,23-dihydro-avermectin B_{1a}	Mice	Doses tested = 0.2, 0.4, 0.8 and 1.6 mg/kg/day. (20/group) Maternal deaths at 0.4, 0.8 and 1.6 mg/kg dose levels. Cleft palate in 10 fetuses from 5 litters at 1.6 mg/kg/day dose level. No control incidence was given. NOEL's: Maternal toxicity = 0.2 mg/kg/day, Developmental = 0.8 mg/kg/day.
22,23-dihydro-avermectin B_{1b}	Mice	Doses tested = 0.4, 0.8 and 1.6 mg/kg/day. (35 in control group; test group sizes were not given) Maternal death (1 per group) at 0.8 and 1.6 mg/kg dose levels. Cleft palate in 10 fetuses from 4 litters at 1.6 mg/kg and 4 fetuses in 4 litters at 0.8 mg/kg dose level. No control incidence was given. NOEL's: Maternal toxicity = 0.4 mg/kg/day, Developmental = 0.4 mg/kg/day.
Ivermectin	Mice	Doses tested = 0.1, 0.2, 0.4, and 0.8 mg/kg/day. (25/group) Maternal deaths at 0.2 (1), 0.4 (3), and 0.8 mg/kg (3). Cleft palate in 3 fetuses from 3 litters at 0.8 mg/kg and 5 fetuses in 4 litters at 0.8 mg/kg dose level. NOEL's: Maternal toxicity = 0.1 mg/kg/day, Developmental = 0.2 mg/kg/day.
Ivermectin	Rats	Doses tested = 2.5, 5, and 10 mg/kg/day. (25/group) 3 dams sacrificed moribund at 10 mg/kg. Cleft palate in 4 fetuses from 2 litters at 10 mg/kg. NOEL's: Maternal toxicity = 5 mg/kg/day, Developmental = 5 mg/kg/day.

Test compound	Species	Results
Ivermectin	Rabbits	Doses tested = 1.5, 3, and 6 mg/kg/day. (16/group) Sedation reported in an unspecified number of does given the 6 mg/kg dose. Decreased maternal body weight was noted, and 6 does aborted in the 6 mg/kg dose group. Cleft palate was seen in 1 fetus in the 3 mg/kg group and in 8 fetuses from 3 litters at 6 mg/kg. Clubbed forepaw was reported in 5 fetuses from 1 litter at 3 mg/kg and in 6 fetuses from 3 litters at 6 mg/kg/day.
Ivermectin	Dogs	Doses tested = 0.5 mg/kg/day (17 in one group treated on gestation days 5, 15, 25 and 35; another group of 19 were treated on gestation days 10, 20, 30 and 40; 17 in a third group served as a control group which was given the sesame oil vehicle on gestation days 5, 10, 15, 20, 25, 30, 35 and 40) No maternal or developmental effects were noted.

3. Studies with Avermectin B_2 in Mice

i. Oral Range-finding Studies (TT-76-723-1/2; undated; MRID No. 00096447)

L-676,897 (lot G05; purity unstated) was suspended in 0.5 % aqueous methyl cellulose.

Groups of 5 mated CF1 mice were administered doses of 0.1, 0.5, or 0.75 mg/kg/day test material in study No. TT-76-723-1 and 1, 2, 4, 6, or 8 mg/kg/day test material in study No. TT-76-723-2, by oral gavage from gestation day 6 through 15.

No mortality was observed at doses ≤ 0.75 mg/kg/day. One death was observed in each group at 1, 2, and 8 mg/kg/day, but no death occured at 4 and 6 mg/kg/day. Death was preceeded by tremors and ensuing coma. Based on the mortality rate observed, a dose-range of 0.1, 0.2, 0.4, and 0.8 mg/kg/day was chosen for 2 replicate definitive developmental studies with Avermectin B_2. The inconsistent death rates observed at doses ≥ 1 mg/kg/day were thought to be related to absorption resulting from use of methylcellulose as a vehicle. Therefore, the vehicle chosen for the definitive studies was sesame oil.

ii. C-076 (B_2: Oral Teratogenic Studies (Replicates Studies No. TT 76-723-0/-3; undated; MRID No. 00096447)

In these two replicated studies, groups (10/group in study No. TT 76-723-0; 15/group in study No. TT 76-723-3) of mated CF1 mice were administered 10 ml/kg sesame oil containing 0, 0.1, 0.2, 0.4, or 0.8 mg/kg/day of test material by oral gavage from day 6 through 15 of gestation. All fetuses from only the control, 0.4 and 0.8 mg/kg/day groups were examined for skeletal alterations. All externally malformed/dead fetuses were also examined, when possible, for visceral/skeletal alterations. Except for maternal body weight/weight gain, the data from both studies were combined to achieve a meaningful number of litters for evaluation.

Mortality was observed in every dose group. Respective death rates were 2, 3, 5, and 5 for the groups treated with 0.1, 0.2, 0.4, and 0.8 mg/kg/day. The affected mice were either found dead or were sacrificed in extremis after 1-2 doses. Death was preceeded by tremors and/or convulsions, then coma. Body weight gain of all surviving mice were unaffected by the test material. Reproductive parameters were also comparable between groups of survivors.

No adverse effects were observed at 0.1 mg/kg/day but a dose-related increase in both the fetal and litter incidences of cleft palate was observed at doses ≥ 0.2 mg/kg/day (0 in controls compared with 2/21, 3/19 and 5/18 litters affected in the 0.2, 0.4 and 0.8 mg/kg/day dose groups, respectively).

Fetal visceral and skeletal alterations were not affected by treatment with the test material.

A maternal NOEL was not established because the LDT (0.1 mg/kg/day) caused 2 deaths. The developmental NOEL was 0.1 mg/kg/day and the LOEL was 0.2 mg/kg/day based on an increase of fetal/litter incidences of cleft palate.

H. Issues and Recommendations

1. A large developmental toxicity data base is available for the avermectin family of compounds. The most characteristic developmental effects of these compounds are cleft palate in mice (LEL = 0.1 mg/kg/day for the 8,9-isomer and 0.4 mg/kg/day for the other members of the class) and reduced viability of rat pups during lactation in postnatal and reproduction studies (LEL = 0.4 mg/kg/day).

2. Avermectin B_1 (an 80:20 mixture of B_{1a} and B_{1b}) did not induce developmental toxicity in the rat at doses ≤ 1.6 mg/kg/day. However, maternal toxicity was not induced in the rat, and

higher doses could have been tested. The analog Ivermectin induced cleft palate in the rat when tested at 10 mg/kg/day. Because the mouse is clearly more sensitive than the rat and will be used for risk assessment purposes, the PRC did not recommend additional testing in the rat.

3. Avermectin B_1 appeared to induce clubbed forefeet in the rabbit at a dose level of 2.0 mg/kg/day. The PRC noted that data were lacking in the study which would allow confirmation of the numbers of normal or affected litters and fetuses. NOELs and LOELs could not be established based on the information available, but the PRC did not recommend additional developmental toxicity testing in the rabbit.

4. In the rat, avermectin B_{1a} caused spastic movements of the limbs of pups during lactation at dose levels $\geq$ 0.2 mg/kg/day in a developmental toxicity study with a postnatal phase. The NOEL for developmental toxicity in this study was 0.1 mg/kg/day. Decreases in pup weights and viability were also observed during lactation in another postnatal study with avermectin B_{1a} at higher dose levels. A postnatal study with the delta 8,9 isomer of avermectin B_1 did not have toxic effects on pups at doses $\leq$ 0.4 mg/kg/day.

5. The delta 8,9-isomer of avermectin B_1 did not induce maternal or developmental toxicity at the highest dose tested (1.0 mg/kg/day) in the rat. The NOEL for maternal toxicity in the mouse was 0.1 mg/kg/day based on increased mortality at doses $\geq$ 0.5 mg/kg/day. The NOEL for the developmental toxicity of this isomer in mice was 0.06 mg/kg/day based on increased incidences of cleft palate at doses $\geq$ 0.1 mg/kg/day. This NOEL was the highest dose tested in one of four studies with the isomer in mice. In the second study, the lowest dose tested (0.05 mg/kg/day) also failed to increase the incidence of cleft palate, while doses of 0.1 and 1 mg/kg/day in the third study and 1.5 mg/kg/day in the fourth study increased the incidence of the effect. The PRC noted that cleft palate is observed with the isomer in the absence of maternal toxicity.

6. The polar degradate of avermectin B_1 did not cause either maternal or developmental toxicity at the highest dose tested in the mouse (1.0 mg/kg/day).

7. In a two-generation reproduction study of avermectin B_1 in rats, the highest dose tested (0.4 mg/kg/day) decreased viability of pups (decreased litter size and increased pup deaths) during lactation days 7 through 21 and decreased pup body weights. The PRC noted that these effects probably resulted from compound ingestion through nursing since the test material was administered by gavage in this study. An

increased incidence of retinal anomalies was observed in F_{2b} pups (the only litters examined histologically) in the 0.4 mg/kg/day dose group. The NOEL for these effects was 0.12 mg/kg/day.

8. The PRC recommended that an RfD_{DT} 0.0006 mg/kg/day be established for purposes of acute dietary risk assessment. This value would be based on the NOEL of 0.06 mg/kg/day established in the mouse developmental toxicity study with the 8,9-isomer (a photodegradation product found in plants) in mice and a 100-fold uncertainty factor. A NOEL of 0.05 mg/kg/day should be used for calculation of margins of exposure for mixer/loader/applicators based on maternal toxicity observed with higher dose levels of the parent compound. The existing RfD of 0.0004 mg/kg/day is derived from the NOEL of 0.12 mg/kg/day in the two-generation rat reproduction study and the use of a 300-fold uncertainty factor. The PRC noted that the greater uncertainty factor is supported by the severity of the effects (pup death) and steep dose-response and should be protective for potential developmental toxicity.

9. The only further testing in the area of developmental and reproductive toxicity recommended by the PRC is a postnatal neurotoxicity study which includes dosing during lactation. The study should be conducted in the rat and dosing by gavage with test material dissolved in oil.