Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 6/16/94
Review Section I, Tox. Branch I
for Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hurley 6/24/94
Review Section I, Tox Branch I

DATA EVALUATION REPORT

STUDY TYPE: 83-6; Developmental Neurotoxicity Study in Rats

TOX. CHEM NO: New Chemical; P.C. Code 122806

MRID NO.: 428515-08

TEST MATERIAL: MK-0244 Technical

SYNONYMS: Emamectin

STUDY NUMBER: TT #91-721-0

SPONSOR: Merck & Co.

TESTING FACILITY: Merck Research Laboratories

TITLE OF REPORT: MK-0244: Oral Developmental Neurotoxicity Study in Female Rats. TT #91-721-0

AUTHOR(S): Dr. David Wise

REPORT ISSUED: April 29, 1993

EXECUTIVE SUMMARY:

Groups of 25 impregnated Sprague-Dawley strain rats were given daily doses of 0, 0.1, 0.6, or 3.6/2.5 mg Emamectin per kg body weight in deionized water beginning on gestation day 6 and continuing through lactation day 20. If an animal did not give birth to a litter on gestation day 24 it was sacrificed and examined to determine pregnancy status. Doses were based on the results of a range-finding study, as well as the results of the pup effects in the 2-generation rat reproduction study.

The progress of pup development was monitored by observation of weekly body weights during and after lactation, and preputial separation was noted on postnatal days (PND) 39, 43, and 47 in male pups. Vaginal canalization was noted in female pups on PNDs 31, 34, and 37.

Selected pups were observed for behavioral changes on PNDs 13, 17, 21, and 59 (±1) for open field motor activity; PNDs 22 and 59 (±1) for auditory startle habituation, and PNDs 24 and 59 for learning and short term retention of passive avoidance and PNDs 32 and 66 for long term retention of learned passive

avoidance.

Other selected pups were sacrificed on PNDs 11 and 60, and body and brain weights were measured. Brain, spinal cord, sciatic nerve, and skeletal muscle tissues from those animals in the control and high dose groups, which were sacrificed on PND 60, were also prepared for microscopic examination.

There were no treatment-related effects on the incidence of clinical observations, the number of implants/dam, the percent postimplantation survival, pup survival during lactation, or the incidence of malformations.

There was an **increase** in maternal body weight gain during gestation at the mid and high dose levels (11 and 15% above controls, respectively). A greater **decrease** in maternal weight gains was observed in the mid and high dose groups during lactation. In the absence of clinical signs, no dose-response relationship (33% and 14% body weight gain decreases at the mid and high dose groups in comparison to controls, respectively), and the stated reason for selecting and reducing the highest dose level in the main study (excessive pup mortality), it is unlikely that the 3.6/2.5 mg/kg/day dose is associated with maternal toxicity.

The NOEL for maternal toxicity is 3.6/2.5 mg/kg/day (highest dose tested).

At the highest dose tested, pup body weights were decreased in comparison to controls by 14 to 41% on lactation days 11, 17, and 21, and body weight gains after weaning were 16 and 17% less than control values for male and female pups, respectively. In addition, clinical signs including head and body tremors, hindlimb extension and splay, and unkempt coat without alopecia were observed in pups from the high dose group during lactation and postweaning. Tremors were noted as early as PND 6 and persisted through PND 27, and hind limb splay appeared in high dose pups as soon as PND 10 and persisted through PND 34.

The developmental landmarks were delayed an average of 3.6 days in both sexes for pups from the high dose.

The behavioral changes were observed primarily in pups given the 3.6/2.5 mg/kg/day dose level. Motor activity was increased in both sexes on PND 13 and decreased in both sexes on PND 17. Motor activity was also decreased in females on PND 59. At the mid dose of 0.6 mg/kg/day, female pups exhibited a dose-related decrease in open field motor activity (activity in mid and high dose females was decreased by 20.6 and 41.2% from controls, respectively, at PND 17. The auditory startle reflex was also decreased in both sexes from the highest dose tested on PNDs 22 and 59.

There were no treatment-related effects in brain weights or neurohistological findings which could be associated with the developmental behavioral results.

The NOEL for developmental neurotoxicity is considered to be 0.10 mg/kg/day (LDT). The LEL is 0.60 mg/kg/day based on the dose-related decrease in open field motor activity in females at PND 17.

Core Classification: **SUPPLEMENTARY**

The study was well conducted with respect to dose selection, and numbers and kinds of observations, but was under reported and a full statistical analyses, as well as individual animal data, were not presented. Additionally, page 25 of the study report, dealing with the decreased open field motor activity of mid and high dose females at PND 17, was missing and is required to be submitted. Also, an explanation of stereotypy time is required. It is noted that there is a 12.5% increase in cerebral cortex measurement in females at the high dose. Individual data were not presented. A 10% change was sustained among females measured on day 58 in brains that were smaller on average. Statistical analysis was not performed. This finding may be an effect of unclear significance.

1. Quality Assurance Statement: A Certification of Good Laboratory Practice was signed by the Study Director, Dr. L. David Wise, and dated April 29, 1993. Quality Assurance Inspections and Audit Dates were signed and dated by members of the Quality Assurance Program.

2. Test Material: MK-0244; Lot Number: L-656,748-052S002; Purity: > 97% based on HPLC; Vehicle: Deionized water;

3. Dosing Suspensions Analyses: Samples of the dosing solutions were assayed for concentration on Study Weeks 1, 4, and 6 and uniformity on Study Weeks 1 and 4.

4. Animals: 100 female Sprague-Dawley rats (Crl:CD™(SD)BR), approximately 11 weeks old, weighing 237 to 353 grams were used in the study. The rats were individually caged prior to and after mating and had free access to pelleted Purina Certified Rodent Chow #5002 and tap water. Females were housed with untreated males of the same strain in a 1:1 ratio. The day of finding plug and/or sperm was considered gestational day 0 (GD 0). on GD 15 to 18, mated females were transferred to individual clear plastic boxes containing dry bedding in preparation of delivery of pups. Maternal animals and pups remained in this type of cage through the lactation period. Room temperature and lighting were under controlled conditions.

5. Methods: Females were assigned to the following groups based on a weight-independent allocation schedule:

Treatment Group	Number of Females per Group
Control	25
MK-0244 at:	
0.1 mg/kg/day	25
0.6 mg/kg/day	25
3.6/2.5* mg/kg/day	25

* between GD 17 and 20 the high-dose level of 3.6 mg/kg/day was reduced to 2.5 mg/kg/day due to the appearance of pup tremors in the 3.6 mg/kg/day group of a concurrent 2-generation rat reproduction study (TT #91-715-0).

Suspensions of MK-0244 in deionized water were prepared daily and kept constantly stirred during dosing. Each female was orally gavaged with a metal catheter once

daily from GD 6 through Lactation Day (LD) 20. Dosing volume was 5.0 ml/kg based on the most recent body weight. Dose selection was based on a range-finding study (TT #90-742-9) in female rats at oral gavage doses of 0.1, 0.7, and 5 mg/kg/day or dietary doses of 1, 7, or 50 ppm of MK-0244 given during GD 0 to LD 21. At the high-dose, there were treatment-related decreases in food consumption and weight gain in dams by both routes of exposure. Developmental toxicity was also seen at the top dose as decreased pup weight, pup tremors and pup death. The high-dose gavage group was euthanized early due to high pup mortality. Neuronal degeneration was observed in high-dose pups at postnatal day (PND) 21. Based on these data, the high-dose of 3.6 mg/kg/day was chosen for this study with the expectation of mild to moderate signs of developmental toxicity. Lower doses were chosen to be 1/6 and 1/36 multiples of the high-dose.

Clinical observations were made daily by at least two persons as follows: 1) With knowledge of treatment group on GD 0 and GD 6 through LD 20, and 2) Without knowledge of treatment groups from GD 6 through sacrifice. Dam body weights were recorded on GD 0, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24; and LD 0, 4, 7, 11, 14, 17, and 21. Parturition was observed and length of gestation measured. Females which delivered were euthanized on LD 23 and 24. Mated females that did not deliver pups were similarly euthanized on GD 24. The uterus of each female was examined and metrial glands counted. Females were then discarded.

6. Type and Frequency of Observations and Analyses of Pups:

(A) Preweaning (Postnatal days (PND) 0 to 31)

All pups were examined daily for clinical signs by two examiners, one of whom did not have knowledge of the treatment schedules. In addition, 1 randomly selected pup/sex/litter was examined for pupillary function once per week from weaning through sacrifice. Pupillary function was assessed in a dimly lit room by shining the beam of a flashlight into the eyes of the hand-restrained animal and observing the closing of the pupil. Body weights were recorded on PND 0, 4, 11, 17, and 21. All pups were examined externally for malformations and sexed on PND 0, 4, 11, 17, and 21. All pups were counted on PND 0, and 10 pups per litter (5 males and 5 females where possible) were selected and identified by a tatoo. On PND 4, each litter was reduced to 4 male and 4 female pups. Remaining non-

selected pups were discarded without further examination. Whenever possible, dead pups were fixed in 10% buffered formalin, but no further examination of dead pups was made. One low-dose euthanized pup, with an abnormal rigidity and posture in the left fore-arm, was examined for skeletal and visceral changes, but except for supernumerary rib, no unusual findings were found.

On PND 11, one male and one female pup, each from different litters, (at least 10/sex/group), were necropsied for removal of the brain. Body and brain weights were recorded. Six brains/sex/group were immersed in neutral 10% buffered formalin. Brains from the control and high-dose groups were prepared by routine methods , stained with hematoxylin and eosin, and examined histologically.

BEHAVIORAL ASSESSMENT

(1) Open Field Motor Activity:

The test was conducted on PND 13, 17, and 21. Computer-controlled monitors were used. Details of the methodology are appended to this report.

(2) Auditory Startle Habituation:

The test was performed on PND 22. The test was designed to assess sensorimotor reflexes and habituation to redundant, nonsignificant stimuli. Two computer-controlled auditory startle chambers were used. Details of the methodology are appended to this report.

(3) Passive Avoidance:

The test was performed on PND 24 and 31. In this test, the pup must learn to inhibit a normally preferred response (i.e., avoiding light by entering a dark compartment) in order to avoid shock. Test 1, which was conducted on PND 24, was designed to assess learning (i.e., response inhibition) and short-term retention; Test 2, conducted 7 days later, was designed to assess long-term retention. Computer-controlled, two-compartment shuttleboxes were used. Details of the methodology are appended to this report.

(B) Postweaning

On PND 23 and 24, 3 to 4 animals per sex were selected from each litter and caged 2/sex/cage under the same

conditions as the dams. All pups were observed daily, as previously described, for clinical signs. Body weights were recorded weekly. Preputial separation of all males was recorded on PND 39, 43, and 47. The presence of vaginal canalization was recorded on all females on PND 31, 34, and 37.

BEHAVIORAL ASSESSMENT

(1) Open Field Motor Activity:

The test was conducted on PND 59 ± 1 with pups that had been previously tested in the preweaning period in the auditory startle habituation test. The test conditions were the same as for the preweaning test.

(2) Auditory Startle Habituation:

The test was conducted on PND 59 ± 1 with pups that had been previously tested in the preweaning period in the passive avoidance test. The test conditions were the same as for the preweaning test.

(3) Passive Avoidance:

The test was conducted on PND 59 ± 1 and 66 ± 1 with pups that had been previously tested in the preweaning period in the open field motor activity test. The test conditions were the same as for the preweaning test, except the level of the 1 sec foot-shock was set at 1 mA, criterion was defined as 3 consecutive trials with ≥ 60 sec latency, and the dark compartment tunnels were removed.

7. Necropsy:

On PND 60, one pup per litter (at least 10/sex/group) were necropsied and body and brain weights recorded. Additionally, 6 animals/sex/group were perfused on PND 59-60 using 4% formaldehyde/1% glutaraldehyde and underwent necropsies limited to removal of brains, spinal cord, optic and sciatic nerves and skeletal muscle. No body or brain weights were taken for perfused rats. Samples of spinal cord, sciatic nerve, optic nerve, and skeletal muscle were processed by routine methods. Further, samples of Gasserian ganglion, dorsal (with ganglion) and ventral roots, and sural, tibial and peroneal branches of sciatic nerve were embedded in plastic. These, sections from control

and high-dose group (the NOEL by paraffin-section assessment) were processed.

All brains were sectioned to include the following structures: cerebral cortex (fronto-parietal, piriform), basal ganglia (nucleus caudatus putamen), hippocampus, thalamic and hypothalamic regions, midbrain (tectum, tegmentum and cerebral peduncles), cerebellum and metencephalon, myelencephalon (brain stem).

Bilateral measurements of cerebral cortical depth (parietal region), hippocampus major and single cerebellar folia (typically in the ansiform, lunate or paraflocculer lobes) were recorded using a micrometer eyepiece at 250X (total magnification) for PND 11 pups and at 25X for PND 59-60 pups.

8. Statistical Analyses:

Statistical analyses were done by an analysis of variance or covariance. A rankit method was used to normalize nonparametric data. Results were considered to be statistically significant if $P \leq 0.05$. A trend analysis was used to determine if there was a significant trend ($P \leq 0.05$) with increasing dosage across all treatment groups. If there was a significant trend ($P \leq 0.05$), data from the high-dose group were excluded and the trend test was repeated. The following parameters were analyzed: Average female body weight changes between GD 6 to 20 and LD 0 to 21 (adjusted for GD 6 and LD 0 weights, respectively); average length of gestation; percent postimplantation survival; average implants per female; percent live pups on PND 0; pup deaths between PND 1-4 and 5-21; average live pup weights on PND 0, 4, 11, 17, and 21 (male and female separately, adjusted for length of gestation and number of live pups where appropriate); average pup postweaning weight gain between postnatal weeks 1 to 7 were analyzed for linear, average, and quadratic time responses (male and female separately); average horizontal activity in the open field motor activity test, average PND 13 stereotypy time in the open field motor activity test, and average V Max in the auditory startle habituation test (male and female separately). Terminal body weight and brain weight data from PND 60 animals were analyzed for normality using the Wilk and Shapiro W statistic, and for homogeneity using the Levene Test. Analysis of variance was by trend test at $P = 0.05$, with rankit transformation where necessary.

RESULTS

There were no deaths or abortions during the study. There were no compound-related clinical signs during gestation or lactation in the dams. During gestation and lactation, the incidence and frequency of the following clinical signs occurred without relationship to treatment: alopecia, laceration, slightly swollen bilateral hindpaws, trace of blood in vagina, regurgitated part of dose, nasal discharge, lethargy/dystocia, scabs, and thorax/axillary masses. There were no treatment-related effects in females with live pups or gestation length. The number of pregnant females with live pups out of the 25 mated females per group was 23, 25, 24, and 25 for the control, low, mid, and high-dose groups, respectively. The mean length of gestation was 22.4, 22.3, 22.4, and 22.5 days for the control, low, mid, and high-dose groups, respectively.

AVERAGE MATERNAL BODY WEIGHT CHANGES (grams)

Dose	Control	Low	Mid	High
Gestation Days				
6 - 12	29 (23)	29 (25)	32 (25)	42 (25)
12 - 20	83	85	91	86
6 - 20	111	113	123	128*
Lactation Days				
0 - 7	23 (22)	23 (25)	22 (24)	21 (25)
7 - 14	13 (23)	17	14	9
14 - 21	-15	-21	-23	-12
0 - 21	21 (22)	19	14	18*

* = Trend is $P < 0.05$ ( ) = number of litters

There were treatment-related increases in gestational weight gain in the mid and high-dose groups during days 6 to 20. The increases were 11 and 15% above control values for the mid and high-dose groups, respectively, but are not considered toxicologically significant.

Additionally, the mid and high-dose groups had statistically significant decreases (33 and 14%, respectively, for the mid and high-dose groups) in lactation weight gain during the 21 day lactation period. Since the results are not dose-related, they are not considered compound-related. The NOEL for maternal toxicity is 3.6/2.5 mg/kg/day (HDT).

SUMMARY OF REPRODUCTIVE STATUS AND WEANING

	CONTROL	LOW	MID	HIGH
Females	23	25	25	25
Implants/Female	16.6	16.6	16.4	16.8
% Postimplantation Survival	88.9	95.0	92.2	92.2
Females with live pups Day 0 Postpartum	23	25	24	25
Females with live pups Day 21 Postpartum	23	25	24	25
Total Pups (M/F)	342(181/160)	398(195/203)	395(184/207)	390(195/195)
Live Pups on Day 0	340(181/159)	394(193/201)	376(176/200)	388(194/194)
Dead Pups on Day 0	2(0/1)	4(2/2)	19(8/7)	2(1/1)
Live Pups/Litter	14.8	15.8	15.0	15.5
% Live Pups	99.4	99.0	95.4	99.5
Live Pups after culling	184	200	192	200
Pup Deaths (% pup deaths)				
Postnatal Days 1 - 4	5(1.3)	7(1.7)	6(1.5)	7(1.9)
Postnatal Days 5 - 11	1(0.5)	0(0.0)	0(0.0)	1(0.5)
Postnatal Days 12 - 17	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Postnatal Days 18 - 21	0(0.0)	0(0.0)	0(0.0)	1(0.5)
Postnatal Days 5 - 21	1(0.5)	0(0.0)	0(0.0)	2(1.0)
Live Female Pup Weight (g)				
Day 0	6.6	6.2	6.5	6.3
Day 4	10.7	10.1	10.4	10.2
Day 11	27.8	26.8	27.6	24.0*
Day 17	46.4	45.2	46.4	33.2*
Day 21	64.9	62.5	63.5	37.9*
Live Male Pup Weight (g)				
Day 0	6.9	6.6	6.8	6.7
Day 4	11.2	10.5	10.9	10.9
Day 11	28.3	27.9	28.3	25.4*
Day 17	47.3	46.6	47.2	35.1*
Day 21	67.1	65.0	65.1	40.3*

* = $P < 0.05$

There were no treatment-related effects in Implants/female, % postimplantation survival, and pup survival. Treatment-related statistically significant decreases in male and female pup body weights were observed at the high-dose on days 11, 17, and 21 of weaning. These decreases ranged from 14 to 41% for both sexes. The NOEL for pup body weight during preweaning is the mid-dose group. There were no compound-related findings with respect to pup (litter) malformations and variations. There were no malformations and the only variations observed were 2 pups (2 litters) with hematoma in the low-dose group.

SUMMARY OF CLINICAL SIGNS OF PUPS IN PREWEANING

		Number of Litters with Signs			
Clinical Sign	Days	Control	Low	Mid	High
No. Litters Exam.		23	25	24	25
Intermittent Head Tremors	6-10	0	0	0	10
Intermittent Body Tremors	7-13	0	0	0	23
Whole Body Tremors	10-25	0	0	0	25
Hindlimb Extension	10-23	0	0	0	25
Hindlimb Splay	15-26	0	0	0	25

There were treatment-related clinical signs in high-dose pups during the preweaning period which consisted of head and body tremors, hindlimb extension and hindlimb splay. The NOEL for clinical signs in pups during preweaning is the mid-dose.

SUMMARY OF CLINICAL SIGNS IN PUPS IN POSTWEANING

Clinical Signs	Days	Control	Low	Mid	High
No. Pups Exam.		161	175	169	174
Unkempt Coat	37-41	0	0	1	6
Hindlimb Splay	24-34	0	0	0	79
Whole Body Tremors	24,27	0	0	0	7
Alopecia	39-41,58-68	1	1	3	4

There were treatment-related clinical signs in postweaning pups at the high-dose, which consisted of unkempt coat, hindlimb splay, and whole body tremors, but not alopecia. The NOEL for pup postweaning clinical signs is the mid-dose.

AVERAGE BODY WEIGHT OF PUPS IN POSTWEANING

	Weeks							
Dose	1	2	3	4	5	6	7	GAIN
FEMALES								
Control	84	136	177	211	242	265	291	207
Low	81	130	171	203	234	255	277	196
Mid	83	132	173	205	236	255	278	195*
High	48	84	127	158	182	199	219	170*
Dose	1	2	3	4	5	6	7	GAIN
MALES								
Control	89	153	224	294	362	408	460	371
Low	88	150	222	293	361	408	459	371
Mid	88	148	220	289	356	404	455	367
High	54	98	157	216	276	319	363	309*

* = $p < 0.05$

There were treatment-related decreases in weight gain during the postweaning period at the high-dose in both sexes which were toxicologically significant. These decreases in weight gain at the high-dose were 16 and 17%, respectively, for males and females. The statistically significant decrease in weight gain at the mid-dose in females was only 5.7% less than controls and was not considered toxicologically significant. The NOEL for pup weight gain during the postweaning period is the mid-dose.

BEHAVIORAL ASSESSMENT

OPEN FIELD TESTING

	MALES				FEMALES			
	C	L	M	H	C	L	M	H
PND 13 NO. PUPS EXAM.	23	25	24	25	23	25	24	25
Mean Horizontal Activity (Beams Interrupted)/Interval								
Average	473	346	398	730*	531	404	539	906*
Mean Stereotypy Time (Sec)								
Average	12	8	8	18*	10	7	9	16*
PND 17 NO. PUPS EXAM.	23	25	24	25	23	25	24	25
Mean Horizontal Activity (Beams Interrupted)/Interval								
Average	1066	1127	889	745*	1147	1097	910*	675*
PND 21 NO. PUPS EXAM.	23	25	24	25	23	24	23	24
Mean Horizontal Activity (Beams Interrupted)/Interval								
Average	719	780	734	887	657	657	658	697

PND 58 to 60

NO. PUPS EXAM.	23	25	23	25	23	25	24	25
Mean Horizontal Activity (Beams Interrupted)/Interval								
Average	1653	1593	1479	1518	1938	1896	1674	1597*

* = $P < 0.05$

Mean horizontal activity and mean stereotypy time were increased on PND 13 in both sexes of pups at the high-dose. Mean horizontal activity was decreased in high-dose males by 30.1% and mid- and high-dose females by 20.6 and 41.2%, respectively, in PND 17. Measurements at PND 21 were comparable between control and treated pups of both sexes and in PND 58 to 60, high-dose females again showed a decrease in average horizontal activity by 17.6%. These findings in the mid- and high-dose groups are considered treatment-related and toxicologically significant. The NOEL for open field motor activity is the low-dose.

AUDITORY STARTLE HABITUATION

	MALES				FEMALES			
	C	L	M	H	C	L	M	H
PND 22 NO. PUPS EXAM.	23	25	24	25	23	25	23	25
Mean V Max (Arbitrary Units)/Block of Trials								
Average	342	348	340	91*	270	291	305	69*
Mean T Max (MSec)/ Block of Trials								
Average	24	25	23	32	22	23	22	27
PND 58 to 60 NO. PUPS EXAM.	23	25	24	25	23	24	23	25
Mean V Max (Arbitrary Units)/Block of Trials								
Average	173	168	169	119*	165	172	178	89*
Mean T Max (MSec)/ Block of Trials								
Average	28	29	28	26	32	32	30	31

* = $P < 0.05$

PND 22 measurements of auditory startle habituation showed that both sexes at the high-dose had 74% decreases in average V Max response across the 50 trial interval. The average T Max response was increased 22-33% above control levels. In the postweaning period, at PND 58 to 60, high-dose males and females had decreased V max responses of 31 and 46%, respectively, without an effect in T Max, in comparison to controls.

The NOEL for auditory startle habituation testing is the mid-dose.

PASSIVE AVOIDANCE TESTING

There were no compound-related effects in treated pups in comparison to controls in Test 1 (measuring learning and short-term retention) at either preweaning (PND 24) or postweaning (PND 59 ± 1) or in Test 2 (measuring long-term retention) at either preweaning (PND 31) or postweaning (PND 66 ± 1). The NOEL for passive avoidance testing is the high-dose. The following results, presented in the report, are shown in the table below:

SUMMARY OF PASSIVE AVOIDANCE TESTING

MALES

		MK-244 MG/KG/DAY		
	CONTROL	**0.1**	**0.6**	**3.6/2.5**
TEST 1 - PND DAY 24				
No. Tested	23	25	24	24
Mean Trial To Criterion	5.0	5.4	5.5	4.9
No. Not Achieving Criterion	1	0	0	0
Mean Latency Trial 1 (Sec)	19.3	10.6	10.2	20.3
No. with Latency in Trial 1	23	25	24	24
TEST 2 - PND 31				
No. Tested	22	25	24	24
Mean Trials to Criterion	3.1	3.7	3.3	3.0
No. Not Achieving Criterion	1	1	0	1
Mean Latency Trial 1 (Sec)	17.6	14.4	16.9	17.7
No. with Latency in Trial 1	16	23	16	21

SUMMARY OF PASSIVE AVOIDANCE TESTING

FEMALES

		MK-244 MG/KG/DAY		
	CONTROL	0.1	0.6	3.6/2.5
TEST 1 - PND DAY 24				
No. Tested	23	24	23	24
Mean Trial To Criterion	4.7	4.8	5.2	4.3
No. Not Achieving Criterion	0	0	0	0
Mean Latency Trial 1 (Sec)	15.8	15.1	18.3	18.9
No. with Latency in Trial 1	23	24	23	24
TEST 2 - PND 31				
No. Tested	23	24	23	24
Mean Trials to Criterion	3.9	3.7	3.7	3.4
No. Not Achieving Criterion	0	0	0	0
Mean Latency Trial 1 (Sec)	15.0	11.4	12.3	16.5
No. with Latency in Trial 1	20	22	23	22

There were no mortalities in pups during the postweaning period.

SUMMARY OF DEVELOPMENTAL SIGNS

Females

	Cont	Low	Mid	High
Vaginal canalization				
Estimated Mean Day of Occurrence	33.7	33.4	33.1	37.4

Males

	Cont	Low	Mid	High
Preputial Separation				
Estimated Mean Day of Occurrence	44.8	44.9	44.8	48.4

Treatment-related postweaning delays in developmental landmarks occurred in high-dose male and female pups. In females, vaginal canalization was delayed 3.7 days at the high-dose in comparison to controls. In males, preputial separation was delayed 3.6 days in the high-dose in comparison to controls. The NOEL for these findings is the mid-dose.

ORGAN WEIGHTS

Due to the 15.6% decrease in males and the 9.7% decrease in female pup body weight of the 11-day old high-dose pups, there was a general decreased developmental effect in these pups which resulted in a decreased absolute, but not relative brain weight. Therefore, it is unlikely that this finding is a brain-specific effect, since relative weights were slightly (10.6%) increased in the high-dose in comparison to controls and there was no dose-response in either sex.

The following table presents the results.

	Control	Low	Mid	High
FEMALES				
Body Weight (grams)	27.7	26.1	26.4	25.0
Brain (grams)	1.09	1.07	1.09	1.05
% B.W.	3.99	4.14	4.16	4.24
MALES				
Body Weight (grams)	28.6	27.4	29.1	24.2
Brain (grams)	1.15	1.10	1.13	1.06
% B.W.	4.06	4.07	3.92	4.49

On postnatal day 60, high-dose pup body weights were decreased by 19-24% in comparison to controls. Absolute brain weights were also decreased in these high-dose pups, but relative brain weights were increased. Therefore, it is unlikely that this finding is a brain-specific effect, since relative weights were significantly (20%) increased in the high-dose in comparison to controls. The following table presents the results.

	Control	Low	Mid	High
FEMALES				
Body Weight (grams)	274	254	253	193*
Brain (grams)	1.92	1.85	1.92	1.73*
% B.W.	0.76	0.73	0.76	0.90*
MALES				
Body Weight (grams)	398	414	393	321*
Brain (grams)	1.98	2.05	2.02	1.90*
% B.W.	0.50	0.50	0.52	0.60*

* = $P < 0.05$

Morphometric Analyses

There were no mensurational differences between the brains of control and high-dose pups on PND 11 or 59-60. The following tables show the means of the particular measurements for PND 11 and 59-60 pups.

PND 11 PUPS

	Control	High-Dose
	Means (Range in Parenthesis)	
Females		
Cerebral Cortex	3.2 (2.8-3.7)	3.6 (2.7-4.7)
Hippocampus	2.8 (2.3-3.4)	2.9 (2.5-3.3)
Cerebellum	1.1 (1.0-1.4)	1.2 (1.0-1.4)
Males		
Cerebral Cortex	3.1 (2.8-3.9)	3.2 (2.8-3.8)
Hippocampus	3.0 (2.7-3.6)	2.9 (2.3-3.3)
Cerebellum	1.2 (0.9-1.5)	1.1 (0.9-1.2)

PND 59-60 PUPS

	Control	High-Dose
	Means (Range in Parenthesis)	
Females		
Cerebral Cortex	4.0 (3.6-4.7)	4.4 (4.1-4.7)
Hippocampus	4.6 (3.8-5.0)	4.7 (4.4-5.1)
Cerebellum	1.9 (1.5-2.5)	1.9 (1.7-2.1)
Males		
Cerebral Cortex	4.2 (3.7-4.8)	4.1 (3.5-4.6)
Hippocampus	5.1 (4.7-5.5)	4.9 (4.2-5.4)
Cerebellum	1.9 (1.5-2.1)	2.0 (1.4-2.6)

Necropsy

There were no treatment-related gross necropsy findings in PND 11 or 59-60 pups.

Histopathology

PND 11 PUPS

Dose	Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
Number Necropsied	12	10	13	12	12	11	11	13
Brain								
Number Examined	6	6					6	6
Not Remarkable	6	6					6	6

PND 59-60 PUPS

Dose	Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
Number Necropsied	16	18	18	18	18	17	19	17
Brain								
Number Examined	6	6					6	6
Not Remarkable	6	6					6	6
Skeletal Muscle								
Number Examined	6	6					6	6
Not Remarkable	6	6					6	5
Focal Degeneration	0	0					0	1
Spinal Cord								
Number Examined	6	6					6	6
Not Remarkable	6	6					6	6

Sciatic Nerve						
Number Examined	6	6			6	6
Not Remarkable	6	6			6	6
Optic Nerve (Eye)						
Number Examined	6	6			6	6
Not Remarkable	6	6			6	6

There were no treatment-related histological findings. The small focus of degeneration in the median vastus lateralis skeletal muscle in one high-dose female pup at PND 59-60 was diagnosed as acute and accompanied by some local edema and neutrophilic infiltration. This change was not considered related to treatment, but rather was compatible with a traumatic event.

Analysis of Dosing Solutions

Analysis of homogeneity low, mid, and high-dose levels showed for samples dated 7/12/91 that the top, middle and bottom analyses for the low-dose were 75, 85, and 82% of target doses; for the mid-dose were 95, 94, and 91% of targeted doses, and for the high-dose were 94, 97, and 94% of targeted doses for the top, middle and bottom of the sample, respectively.

Analysis of homogeneity low, mid, and high-dose levels showed for samples dated 7/31/91 that the top, middle and bottom analyses for the low-dose were 73, 74, and 76% of target doses; for the mid-dose were 88, 93, and 87% of targeted doses, and for the high-dose were 95, 93, and 91% of targeted doses for the top, middle and bottom of the sample, respectively.

Summary of concentration analyses of samples dated 8/15/91 showed averages of 82, 89, and 92% for the low, mid, and high-dose levels, respectively.

Results of storage stability sample analysis showed that for water fortified with 0.11 mg/ml of MK-0244,

recoveries from control water averaged 95% throughout the course of the study. For a fortification level of 5.23 mg/ml MK-0244, recoveries from control water average 105.5% throughout the course of the study.

DISCUSSION

The study was well conducted with respect to dose selection, number and kinds of observations, but was under reported and a full statistical analyses, as well as individual animal data, were not presented. Additionally, page 25 of the study report was missing and is required to be submitted. Also, an explanation of stereotypy time is required. There is a 12.5% increase in cerebral cortex measurement in females at the high dose. Individual data were not presented. A 10% change was sustained among females measured on day 58 in brains that were **smaller** on average. Statistical analysis was not performed. This finding may be an effect of unclear significance.

The maternal NOEL is 3.6/2.5 mg/kg/day (HDT). At the mid-dose, 0.6 mg/kg/day, there was a 33% decrease in weight gain during lactation. Although the decrease in weight gain at the high-dose was 14% during lactation, the lack of clear dose-response negates the findings at the mid-dose.

The NOEL for pup toxicity, based only on open field motor activity, is the low-dose of 0.10 mg/kg/day. Mean horizontal activity was decreased in high-dose males by 30.1% and mid- and high-dose females by 20.6 and 41.2%, respectively, in PND 17. These findings in the mid- and high-dose groups are considered treatment-related and possibly toxicologically significant. Page 25 of the Study Report relating to these measurements was a blank page in the submitted document.

There were no treatment-related effects in Implants/female, % postimplantation survival, and pup survival. Treatment-related statistically significant decreases in male and female pup body weights were observed at the high-dose on days 11, 17, and 21 of weaning. These decreases ranged from 14 to 41% for both sexes. The NOEL for pup body weight during preweaning is the mid-dose group. There were no compound-related findings with respect to pup (litter) malformations and variations. There were no malformations and the only variations observed were 2 pups (2 litters) with hematoma in the low-dose group.

There were treatment-related clinical signs in high-dose pups during the preweaning and postweaning periods which consisted of head and body tremors, hindlimb extension, hindlimb splay, and unkempt coat (postweaning). The NOEL for clinical signs in pups during preweaning and postweaning periods is the mid-dose.

There were treatment-related decreases in weight gain during the postweaning period at the high-dose in both sexes which were toxicologically significant. These decreases in weight gain at the high-dose were 16 and 17%, respectively, for males and females. The statistically significant decrease in weight gain at the mid-dose in females was only 5.7% less than controls and was not considered toxicologically significant. The NOEL for pup weight gain during the postweaning period is the mid-dose.

Mean horizontal activity and mean stereotypy time were increased on PND 13 in both sexes of pups at the high-dose. Mean horizontal activity was decreased in high-dose males by 30.1% and mid- and high-dose females by 20.6 and 41.2%, respectively, in PND 17. Measurements at PND 21 were comparable between control and treated pups of both sexes and in PND 58 to 60, high-dose females again showed a decrease in average horizontal activity by 17.6%. These findings in the mid- and high-dose groups are considered treatment-related and toxicologically significant. The NOEL for open field motor activity is the low-dose.

PND 22 measurements of auditory startle habituation showed that both sexes at the high-dose had 74% decreases in average V Max response across the 50 trial interval. The average T Max response was increased 22-33% above control levels. In the postweaning period, at PND 58 to 60, high-dose males and females had decreased V max responses of 31 and 46%, respectively, without an effect in T Max, in comparison to controls. The NOEL for auditory startle habituation testing is the mid-dose.

There were no compound-related effects in treated pups in comparison to controls in Test 1 (measuring learning and short-term retention) at either preweaning (PND 24) or postweaning (PND 59 ± 1) or in Test 2 (measuring long-term retention) at either preweaning (PND 31) or postweaning (PND 66 ± 1). The NOEL for passive avoidance testing is the high-dose.

There were no mortalities in pups during the postweaning period.

Treatment-related postweaning delays in developmental landmarks occurred in high-dose male and female pups. In females, vaginal canalization was delayed 3.7 days at the high-dose in comparison to controls. In males, preputial separation was delayed 3.6 days in the high-dose in comparison to controls. The NOEL for these findings is the mid-dose.

Due to the 15.6% decrease in males and the 9.7% decrease in female pup body weight of the 11-day old high-dose pups, there was a general decreased developmental effect in these pups which resulted in a decreased absolute, but not relative brain weight. Statistical analysis was not performed. This finding may be an effect of unclear significance. Therefore, it is unclear whether or not this finding is a brain-specific effect, since relative weights were slightly (10.6%) increased in the high-dose in comparison to controls and there was no dose-response in either sex.

On postnatal day 60, high-dose pup body weights were decreased by 19-24% in comparison to controls. There is a 12.5% increase in cerebral cortex measurement in females at the high dose. Individual data were not presented. A 10 % change was sustained among the females measured on day 58 in brains that were **smaller** on average. Absolute brain weights were also decreased in these high-dose pups, but relative brain weights were increased. Therefore, it is uncertain whether or not this is an effect of unclear significance. The study author considers this finding unlikely to be a brain specific-effect, since relative weights were significantly (20%) increased in the high-dose in comparison to controls.

There were no mensurational differences between the brains of control and high-dose pups on PND 11 or 59-60.

There were no treatment-related gross necropsy findings in PND 11 or 59-60 pups.

There were no treatment-related histological findings. The small focus of degeneration in the median vastus lateralis skeletal muscle in one high-dose female pup at PND 59-60 was diagnosed as acute and accompanied by some local edema and neutrophilic infiltration. This change was not considered related to treatment, but rather was compatible with a traumatic event.

Enforcement Review

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