DATA EVALUATION RECORD

GLYPHOSATE TRIMESIUM

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300
MRID 45539801 (main study), 45539802 (range-finding), 45539803 (range-finding)

Prepared for

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Office of Pesticide Programs
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STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 128501

DP BARCODE: D285565
SUBMISSION NO.: S621853

TEST MATERIAL (PURITY): Technical Grade Glyphosate trimesium (57.4%)

SYNONYMS:

CITATION: Moxon, M.E. (2001) Glyphosate trimesium: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Laboratory study number RR0823; October 19, 2001. MRID 45539801. Unpublished

Moxon, M.E. (1999) Glyphosate trimesium: Second preliminary developmental neurotoxicity study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Laboratory study number RR0826; August 27, 1999. MRID 45539802. Unpublished

Moxon, M.E. (1999) Glyphosate trimesium: Preliminary developmental neurotoxicity study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Laboratory study number RR0817; August 27, 1999. MRID 45539803. Unpublished

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EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2001, MRID 45539801), Glyphosate trimesium (57.4% a.i., batch # P11) was administered to 30 female Wistar-derived rats/group by gavage at doses of 0, 10, 25, or 100 mg (a.i.) /kg/day from gestation day 7 through postnatal day (PND) 11. Doses were based on the results of two range-finding studies (1999, MRIDs 45539802, 45539803). A Functional Operational Battery (FOB) was performed on all dams on gestation days 10 and 17 and on lactation days 2 and 9. On postnatal day 5, litters were culled (when possible) to yield four males and four females. Offspring, from at least 20

litters/dose, were allocated for detailed clinical observations (FOB), assessment of motor activity, auditory startle response habituation, and learning and memory. Neural tissues were collected (10/sex/dose) on PND 12 and at study termination (63 days of age). Pup physical development was assessed by body weight and sexual maturation was assessed for females by age at vaginal opening and for males was by age at completion of balano-preputial separation.

Maternal Toxicity. No treatment-related effects were seen in the dams on survival, clinical signs, FOB, or reproductive performances at any dose level. The salivation and the decreases in body weight, body weight gains or food consumption seen at the high dose (100 mg/kg/day) were not considered to be adverse treatment-related effects. **The maternal LOAEL is greater than 100 mg/kg/day; the highest dose tested. The maternal NOAEL was not established.**

Developmental Toxicity. Overall motor activity was decreased in males and females at the 25 and 100 mg/kg/day dose groups on lactation day 14. Activities were decreased to 72% and 65% for the mid-dose males and females, respectively, and to 70% and 45% for the high-dose males and females, respectively. At day 18, in females activities were reduced 60% in the mid dose group but only 34% in the high dose group. At 100 mg/kg/day there was a decrease (19%) in survival on Day 5 and during Days 1-5, the percentage of pup survival was lower (84.8%) than controls (93%). At 100 mg/kg/day, body weight gains for males and females were reduced approximately 10% during lactation days 1-5. **The offspring LOAEL is 25 mg/kg/day, based on dose-dependent decreases in motor activity in males and females on PND 14. The offspring NOAEL is 10 mg/kg/day.**

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of learning and memory in the offspring and the pending review of the positive control data.

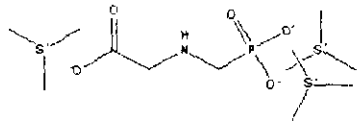
COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Technical grade Glyphosate trimesium (trimesium = trimethylsulfonium ion).

Description:	Pale yellow liquid
Lot/Batch #:	P11
Purity:	57.4 % a.i.
Compound Stability:	Confirmed June 2000
CAS # of TGAI:	
Structure:	



2. **Vehicle and/or positive control:** Water was used as the vehicle and negative control. No positive control was used in this study.

3. **Test animals (P):**

Species:	Rat
Strain:	Alpk:AP$_f$SD
Age at study initiation:	Females: 10-12 wks
Wt. at study initiation:	200-288 g
Source:	Rodent Breeding Unit (RBU), Alderley Park, Macclesfield, Cheshire
Housing:	Individually or with litter in solid-bottomed cages with nesting material; F_1 animals 4/sex after weaning.
Diet:	Powdered CT1 diet, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22±3°C **Humidity:** 30-70% **Air changes:** at least 15/hour **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	6 Days before start of dosing

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: January 10, 2000; End: September 6, 2000

2. **Study schedule:** Time-mated females were delivered to the testing facility and assigned to study. The test substance was administered to the maternal animals from gestation day 7 through lactation day 11. Pups were weaned on postnatal day 29, after which time maternal

animals were killed. The study included 30 parent females/dose level. F1 pups remained on study up to postnatal day 63 (study termination).

3. **Mating procedure:** Mating was carried out at the Rodent Breeding Unit prior to delivery of the animals to the testing facility. The day on which sperm were detected in a vaginal smear was designated gestation day 1.

4. **Animal assignment:** Mated females and offspring were allocated as shown in Table 1. The study had a replicate (randomized block) design. Each replicate consisted of one cage/animal per treatment group. Cages were allocated to each group using an automatic method. The parental females were randomly allocated to cages/treatment groups on arrival at the testing facility. Litters of 7 or 8 pups with at least 3 male and 3 females pups were used for selection of the F_1 generation.

   One pup/sex/litter/group was allocated on postnatal day 5 to one of the following: motor activity, acoustic startle habituation, water maze, detailed observational battery, and sacrifice and brain examination on postnatal day 12. On day 63, 10 animals/sex/group were sacrificed by perfusion and neural and muscle tissues collected for microscopic examination.

TABLE 1. Study design

Experimental parameter	Dose (mg/kg/day)*			
	0	10	25	100
Maternal animals–Main study				
	No. of animals assigned			
FOB (GD 10, 17; LD 2, 9)	All	All	All	All
Offspring– Main study				
Motor activity (PND 14, 18, 22, 60)	10/sex	10/sex	10/sex	10/sex
Acoustic startle habituation (PND 23,61)	10/sex	10/sex	10/sex	10/sex
Passive Avoidance	not tested			
Detailed clinical/FOB (PND 5, 12, 22, 36, 46, 60)	10/sex	10/sex	10/sex	10/sex
Water maze (PND 21 and 59, 3 days after first test)	10/sex	10/sex	10/sex	10/sex
Gross necropsy and Brain Measurements (PND 12, 63)	10/sex	10/sex	10/sex	10/sex

*as active ingredient glyphosate trimesium adjusted for purity.

5. **Dose selection rationale:** Dose levels were chosen based on the results of two range-finding studies in Alpk:AP$_f$SD rats (1999, MRIDs 45539802, 45539803). Details of these studies are given in the appendix. Briefly, groups of 10 female rats were administered by gavage 0, 25, 50, 100, 133, 166, or 200 mg/kg/day from GD 7 through lactation day 11. Doses of 133 mg/kg/day and higher were excessively toxic to dams and pups including maternal and post natal pup mortality. At 100 mg/kg/day maternal body weight and food consumption was slightly reduced during gestation and lactation. No effects on any litter parameter were noted at 25, 50, or 100 mg/kg/day. Based on these results, the doses selected for the developmental

neurotoxicity study were 0, 10, 25, and 100 mg/kg/day. Although only minimal maternal toxicity was observed in the range-finding studies at 100 mg/kg/day, the steepness of the dose-response curve precluded evaluation of higher doses without the risk of maternal death and post natal pup mortality.

6. **Dosage administration:** The test article was administered to parent female rats once daily by gavage from GD 7 through lactation day 11, inclusive. A dose volume of 1 mL/100 g (or 10 mL/kg) was used based on the individual daily body weight.

7. **Dosage preparation and analysis:** Dosing solutions were prepared weekly and stored at room temperature. For each dose level, an appropriate amount of water was added to a weighed amount of test substance. Homogeneity was determined in samples from the top, middle, and bottom of the low- and high-dose solutions from the first batch prepared. Stability was measured in low- and high-dose solutions under the conditions of storage used, for at least the period of use. Concentrations of each dosing solution were analyzed prior to use.

Results:
Homogeneity analysis: Concentrations of samples from the low- and high-dose solutions were 99-100% and 99-102%, respectively of nominal.

Stability analysis: After 12 days of storage at room temperature, concentrations of the low- and high-dose solutions were 100 and 99%, respectively, of their initial measured concentrations.

Concentration analysis: Absence of test article was confirmed in the vehicle. Throughout the study, concentrations of the low-, mid-, and high-dose solutions were 100-103%, 98.4-101.2%, and 100-102%, respectively, of nominal.

The analytical data indicated that the mixing procedure was adequate and that the actual dosage to the animals was acceptable.

C. OBSERVATIONS

1. **In-life observations:**

a. **Maternal animals:** Detailed clinical observation were recorded daily and prior to dosing on each dosing day. Cage-side observations were made as soon as possible after dosing and towards the end of each working day.

All females were examined outside the home cage on GD 10 and 17 and lactation days 2 and 9. It was not stated whether the observers were blind to the animal's treatment group. In addition, the method of ranking was not stated and severity scores were described only as slight (s) or moderate (m). The following functional observations were recorded.

	Functional observations–Maternal animals
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Presence or absence of piloerection and exophthalmos, 3) Urination and defecation 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded on arrival, GD 4, daily prior to dosing from GD 7 through lactation day 11, and on lactation days 12, 15, 22, and 29. Food consumption was recorded at three day intervals during gestation and weekly during lactation. Only food consumption data for the gestation interval were included in the report.

b. Offspring:

1. **Litter observations:** Each litter was examined as soon as possible after completion of parturition (lactation day 1 or PND 1). On days 1 and 5, the sex, weight, and clinical condition of each pup were recorded. Litters were checked daily throughout lactation for dead or abnormal pups.

   On day 5 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded.

2. **Developmental landmarks:** Beginning on postnatal day 41, male offspring were examined daily for preputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset was recorded and the animals were weighed.

3. **Detailed observations:** Offspring were examined for clinical signs and survival once daily during lactation. From lactation day 5, detailed clinical observations were recorded at the same time the rats were weighed. Individual offspring body weight data were recorded within 24 hours of birth, on lactation days 5, 12, 18, and 22, and once weekly thereafter.

4. **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.

i). **Functional observational battery (FOB):** On postnatal days 5, 12, 22, 36, 46, and 60, approximately 10 offspring/sex/group (one male or one female from each litter) were examined outside the home cage in an FOB assessment. It was not stated whether the

observers were blind to the animal's treatment group. In addition, the method of ranking was not stated and severity scores were described only as slight (s) or moderate (m).

FUNCTIONAL OBSERVATIONS- Offspring	
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Presence or absence of piloerection and exophthalmos, 3) Urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

ii). Motor activity testing: Motor activity was evaluated in 10 rats/sex/dose on days 14, 18, 22, and 60. The report stated only that "the test was conducted in a separate room to minimize disturbance and used automated activity recording apparatus which recorded small and large movements as an activity count." Each test was divided into 10 scans of five minutes each. No description of background noise or light intensity was provided.

iii). Auditory startle habituation: Auditory startle reflex habituation testing was performed on 10 offspring/sex/dose on postnatal days 23 and 61 using an automated system. The mean response amplitude and the time to maximum amplitude on each block of 10 trials (5 blocks of 10 trials per session) were calculated. No description of the startle stimulus was given.

iv). Learning and memory testing:

WATER MAZE: Learning and memory testing was performed in 10 offspring/sex/dose Y-water maze with one escape ladder. The time to find the escape ladder was recorded for each trial. The pups were given 6 initial trials on each of days 21 and 59 and three days later were retested using the same procedures. The position of the escape ladder was different for the trials done at the earlier time point to those conducted later. In addition each animal was placed in a straight channel immediately after the six trials in the Y-maze to evaluate swimming speed. No further description of the testing apparatus was given. However, a recently submitted positive control study (2003, MRID # 46012924) from this laboratory described the apparatus and methods used.

5. Postmortem observations:

i). **Maternal animals:** Animals were sacrificed with halothane Ph. Eur. vapor followed by exsanguination and subjected to gross examination. Females which failed to litter were sacrificed on day 26, those with litters not required for selection of F_1 animals were killed on lactation day 5, and the remaining females on study were sacrificed on day 29.

ii). **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 12 or 63. These animals were subjected to postmortem examinations as described below.

PND 12: At postnatal day 12, 10 pups/sex/dose were sacrificed for gross necropsy and brain weight measurements. Animals were sacrificed by carbon dioxide inhalation and the brain immediately removed and fixed in 10% neutral buffered formol saline. The brains were weighed after approximately 24 hours fixation. The brains from six of these animals/sex in the control and high-dose group were processed for neuropathological examination.

After the gross brain measurements were recorded, brains from control and high-dose rats were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Seven coronal sections from control and high-dose animals were examined microscopically. Brain morphometric measurements were made using a KS400 image analysis system.

The following brain morphometric measurements were made for control and high-dose animals:

Caudal edge of olfactory bulb (height and width)
Dorsal cortex parallel to midsaggital line (inner origin at most dorsal point of external capsule)
Dorsal cortex (inner origin at a point cut by a line drawn diagonally to midsaggital line)
Pyriform cortex at midpoint between rhinal and amygdaloid fissures
Hippocampus from midline to outer edge of most lateral pyramidal cells or from widest point from inner zone of dentate gyrus to outer edge of CA2
Dorsal cortex measurement made at right angles to a tangential line at surface of brain to run through the medial tip of the dentate gyrus
Thalamic height and width
Width of cerebral cortex and thalamus at widest point
Width of dentate gyrus at level of most medial part of lower limb of CA3 or widest point
Length of dentate gyrus
Cerebellum (height and length)
Preculminate and prepyrimidal fissures (thickness of various layers)

PND 63: Ten animals/sex/group were euthanized by carbon dioxide asphyxiation, subjected to gross necropsy and the brains were removed, weighed (fresh weight), and discarded. Another 10 rats/sex/dose were anesthetized by injection of sodium pentobarbitone and killed by perfusion fixation with a modified Karnovsky's fixative. The brain (weighed), vertebral column, spinal cord, peripheral nerves (sciatic, sural, and tibial), gasserian ganglion, and gastrocnemius muscle were preserved in an appropriate fixative.

The brain and muscle were embedded in paraffin wax, sectioned at 5 µm, and stained with H&E. Transverse sections of the vertebral column containing samples from the lumbar and cervical regions of the spinal cord, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax, sectioned at 5 µm, and stained with H&E. The remaining tissues were embedded in ARALDITE® and semi-thin sections cut and stained with toluidine blue.

The brain of 6 rats/sex from the control and high-dose groups were examined at seven levels. Spinal cord sections from the cervical and lumbar regions were examined in transverse section. Spinal roots and dorsal root ganglia from C3-C6 and L1-L4 levels and the Gasserian ganglia were examined. Transverse and longitudinal sections of the sciatic nerve and transverse sections of the sural and tibial nerves were examined. The gastrocnemius muscle was examined in transverse plane. Detailed morphometric evaluation was conducted as described for day 12 animals.

D. DATA ANALYSIS:

1. **Statistical analyses:** Body weights were analyzed by ANCOVA using GD 7 or lactation day 1 body weights for dams or lactation day 1 or 5 body weights of pups as the covariate. Food consumption during gestation, litter size, initial (day 1) pup weight, and total litter weight were analyzed by ANOVA. Proportion data were considered by Fisher's exact test. Percentages were analyzed by ANOVA followed by the double arcsine transformation of Freeman and Tukey.

   Motor activity measurements, acoustic startle peak amplitude data, time to complete straight channel water maze, and mean day for attainment of developmental landmarks were analyzed by ANOVA. The mean time per water maze trial was analyzed by Student's t-test. Brain weight and morphology data were considered by ANOVA and ANCOVA on final body weight.

2. **Indices:** Reproductive and offspring viability indices were not calculated. Proportions and percentages for group totals were given for pups live born, pup survival days 1-5, and sex distribution.

3. **Positive and historical control data:** Actual positive and historical control data are not presented; however, comments stating that positive control studies have been previously performed are presented. References for these positive control studies are presented on p. 37 of MRID 45539801. These studies are under review.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No treatment-related maternal deaths occurred before scheduled termination. The number of females found to have insufficient pups was 3, 5, 6, and 8 in the control, low-, mid-, and high-dose groups, respectively. (Sufficient pups were defined as at least 3 males and 3 females in a litter of at least 7 pups.)

   Slight salivation was observed in 22/30 high-dose animals between GD 17 and approximately lactation day 11 and in 3/30 mid-dose animals during lactation days 3-11. No other treatment-related clinical signs of toxicity were seen during general observations or the FOB.

2. **Body weight and food consumption:** Group mean absolute body weights and body weight gains for pregnant or nursing dams for selected intervals are summarized in Table 2. Statistical analyses were conducted on adjusted weight data (not shown in Table 2) not absolute body weight data.

During gestation, body weights adjusted for GD 7 weight were significantly ($p \leq 0.05$ or 0.01) reduced in the high-dose group on GDs 8 and 11-21. This was due to a weight loss following the first day of dosing and weight gains by the high-dose group reduced by 18% during the first week of dosing. No treatment-related differences in body weights were noted between the treated and control groups during lactation.

Food consumption by the high-dose dams was decreased to 84-92% ($p \leq 0.05$) or was 8 to 16% less than controls beginning with the GD 7-10 interval and continuing throughout gestation.

TABLE 2. Selected maternal body weight (mean±SD; g) and weight gain (g) data during gestation and lactation				
Observation/study interval	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Gestation (n= 29-30)				
Body wt. GD 1	247.7 ± 19.4	253.1 ± 15.5	249.7 ± 15.8	254.3 ± 17.3
Body wt. GD 7	276.9 ± 22.4	279.3 ± 19.8	277.8 ± 17.4	280.8 ± 18.3
Body wt. GD 8	278.7 ± 22.2	283.3 ± 20.4	279.1 ± 17.6	279.8 ± 18.3
Body wt. GD 15	316.5 ± 23.1	318.4 ± 20.6	317.2 ± 20.4	313.4 ± 18.8
Body wt. GD 22	392.7 ± 28.0	394.1 ± 27.8	396.4 ± 26.6	393.0 ± 21.1
Wt. gain GD 7-8[a]	1.8	4.0	1.3	-1.0
Wt. gain GD 7-15[a]	39.6	39.1	39.4	32.6 (118%)[b]
Wt. gain GD 15-22[a]	76.2	75.7	79.2	79.6
Lactation (n=16-24)				
Body wt. lactation day 1	301.7 ± 33.4	298.0 ± 26.3	308.1 ± 25.6	305.0 ± 20.1
Body wt. lactation day 5	318.3 ± 24.1	326.2 ± 23.9	333.7 ± 23.5	321.8 ± 19.8
Body wt. lactation day 11	333.8 ± 31.0	334.1 ± 18.5	354.6 ± 23.3	344.0 ± 19.2
Body wt. lactation day 29	335.5 ± 25.5	339.8 ± 21.9	348.9 ± 23.9	343.9 ± 22.0
Wt. gain days 1-5[a]	16.6	28.2	25.6	16.8
Wt. gain days 5-29[a]	17.2	13.6	15.2	22.1

Data taken from Table 6, pp. 76-80, MRID 45539801.
[a]Calculated by reviewer from group means.
[b]Number in parentheses is percent of control: calculated by reviewer.

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. No treatment-related effects were noted for the number of animals in each group that delivered litters with live pups.

TABLE 3. Reproductive performance				
Observation	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Number Mated	30	30	30	30
Number Delivered	30	30	29	29

Data taken from Table 8, p. 82, MRID 45539801.

4. **Maternal postmortem results:** Gross necropsy of dams was unremarkable.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation days 1-5 are summarized in Table 4. Mean litter data were not given for the post-cull interval.

Reduced pup survival from high-dose litters was apparent as significantly ($p \leq 0.05$ or 0.01) decreased numbers of pups/litter on day 5 and percentage of pups surviving days 1-5. The total number of pups missing and presumed dead was increased in the high-dose group during days 1-5 (65 pups vs. 37 control pups); after lactation day 5 the number of pups missing was slightly increased for high-dose males (6 vs 0 controls), but females were not affected. No effects were observed on the number of pups born live or the sex ratio at birth. Whole litter loss after day 1 occurred in one control, one mid-dose, and two high-dose dams.

During lactation days 1-5, a large number of high-dose pups were found to be cold to the touch (75 pups from 11 litters vs. 1 pup from a control litter). Two high-dose female pups were sacrificed moribund on lactation day 12 after the observations of thinness, coldness to touch, dehydration, and decreased activity. No other treatment-related clinical signs of toxicity were observed in pups during lactation.

TABLE 4. Litter size and viability

Observation	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
Number of Litters	30	30	29	29
Dams with whole litter loss	1	0	1	2
Litters with 7 or more pups.	26	25	23	20
Total number born	376	369	342	338
Number born live	373	364	337	330
Number born dead	3	5	5	8
Mean No. of viable pups[a] Day 1 Day 5 (pre-cull)	 12.4 ± 2.1 11.6 ± 2.3	 12.1 ± 3.6 11.3 ± 3.7	 12.0 ± 2.9 10.9 ± 2.7	 11.3 ± 2.6 9.4** ± 2.5
Sex ratio day 1 (%)	54.1	51.0	47.6	56.3
Percentage of pups surviving days 1-5	93.0	92.8	92.1	84.8*
Number pups missing/presumed dead days 1-5 (male + female)	37	24	31	**65**
Number pups missing/presumed dead day 12[b]: male female	 0 1	 0 1	 2 2	 6 2

Data taken from Tables 8-12, pages 82-86, and Table 15, p. 90, MRID 45539801.
[a]Excludes whole litter losses.
[b]Day 12 was the only day deaths were listed.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

2. **Body weight:** Selected mean preweaning pup body weight data are presented in Table 5. Absolute body weights for high-dose female pups were slightly, but significantly ($p \leq 0.05$) less than the controls on lactation day 1. On day 12, body weights adjusted for day 5 weight were significantly ($p \leq 0.05$) less for the high-dose males and females. Body weight gains for the high-dose males and females were reduced approximately 10% during lactation days 1-5

but comparable to the controls thereafter. No treatment-related effects on body weight or body weight gain were noted in low- or mid-dose pups.

TABLE 5: Offspring body weights and body weight gains (g) during lactation				
Lactation day	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Males				
1	5.8 ± 0.5	5.8 ± 0.7	5.9 ± 0.5	5.6 ± 0.5
5 (pre-cull)	8.7 ± 1.2	8.8 ± 1.2	8.7 ± 1.2	8.2 ± 1.3
5 (post-cull)	8.8 ± 1.0	8.9 ± 1.1	8.7 ± 1.1	8.4 ± 1.1
12	22.1 ± 2.9	22.2 ± 1.9	22.1 ± 2.9	19.8 ± 3.0 (↓10)**
18	36.3 ± 4.1	36.7 ± 2.7	37.1 ± 3.6	35.9 ± 4.6
22	49.3 ± 5.2	49.5 ± 3.8	49.7 ± 5.6	48.6 ± 5.4
29	89.3 ± 7.3	88.8 ± 5.2	90.7 ± 6.7	87.4 ± 8.5
Wt. gain days 1-5[b]	2.9	3.0	2.8	2.6
Wt. gain days 5-29[b]	80.5	79.9	82.0	79.0
Females				
1	5.5 ± 0.6	5.5 ± 0.7	5.5 ± 0.5	5.2* ± 0.5 (↓5)
5 (pre-cull)	8.4 ± 1.2	8.3 ± 1.1	8.3 ± 1.2	7.8 ± 1.2
5 (post-cull)	8.4 ± 1.1	8.3 ± 1.1	8.3 ± 1.2	8.0 ± 1.0
12	21.1 ± 2.7	21.3 ± 1.7	21.2 ± 3.1	18.5 ± 3.5 (↓12)
18	34.8 ± 3.8	35.2 ± 2.3	35.6 ± 3.9	34.6 ± 4.4
22	47.3 ± 4.6	47.6 ± 3.2	47.6 ± 5.7	47.2 ± 5.5
29	83.9 ± 6.3	83.5 ± 4.5	84.1 ± 9.0	82.3 ± 7.5
Wt. gain days 1-5[b]	2.9	2.8	2.8	2.6
Wt. gain days 5-29[b]	75.5	75.2	75.8	74.3

Data taken from Tables 13 and 17, pp. 87-88 and 127-130, respectively, MRID 45539801.
[a]Numbers in parentheses are percent of control; calculated by reviewer.
[b]Calculated by reviewer from group means.
Significantly different from control: *$p \leq 0.05$.

Body weights of the males and females from the treated groups were comparable to the controls throughout the post weaning interval (Table 6). Body weights adjusted for day 5 weight for the mid-dose males were significantly ($p \leq 0.05$) *greater* than those of the controls beginning on day 43. A similar trend was not seen in females.

TABLE 6: Offspring body weights (g) during the post weaning interval				
Age (days)	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
Males				
36	138.4 ± 10.1	137.8 ± 6.9	141.1 ± 12.6	135.1 ± 11.5
43	191.2 ± 13.4	192.4 ± 9.6	197.5 ± 15.5*	188.8 ± 15.3
50	245.0 ± 15.6	246.6 ± 11.7	251.8 ± 18.0*	240.9 ± 19.9
57	298.6 ± 18.8	300.1 ± 12.8	306.4 ± 19.6*	294.9 ± 21.5
63	333.9 ± 22.8	335.0 ± 16.6	341.3 ± 21.2*	330.1 ± 24.2
Females				
36	119.7 ± 7.9	120.4 ± 7.7	121.5 ± 10.7	119.7 ± 9.6
43	153.7 ± 8.7	154.9 ± 7.7	156.8 ± 11.1	153.7 ± 10.9
50	176.7 ± 10.3	179.5 ± 9.2	180.5 ± 11.1	178.7 ± 12.5
57	197.8 ± 12.9	200.9 ± 9.6	201.0 ± 11.2	200.8 ± 12.5
63	206.3 ± 15.3	209.8 ± 10.0	209.3 ± 13.3	210.2 ± 13.1

Data taken from Table 17, pp. 127-130, MRID 45539801.
Significantly different from control: *$p \leq 0.05$.

3. Developmental landmarks:

a. Sexual maturation: No treatment-related effects on the mean age for attainment of preputial separation for males or vaginal opening for females were observed. The data are presented in Table 7. Mean body weights of males and females at the day of attainment were similar between the treated and control groups with the exception of mid-dose males which were significantly greater (~3%) than the controls.

TABLE 7. Mean (±SD) age of sexual maturation (days)				
Endpoint	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
N (M/F)	26/26	25/25	22/22	19/19
Preputial separation (males)	45.3 ± 1.3	45.1 ± 1.1	45.7 ± 1.6	45.6 ± 1.2
Vaginal opening (females)	35.8 ± 1.7	35.7 ± 2.0	35.3 ± 1.8	35.9 ± 1.8

Data taken from Table 18, pp. 131-132, MRID 45539801.

4. Behavioral assessments:

a. Functional observational battery: No treatment-related effects were observed at any dose level on any test day (PND 5, 12, 22, 36, 46, or 60).

b. Motor and locomotor activity: Total motor activity counts are given in Table 8; data for motor and locomotor activity were not separated.

TABLE 8: Total mean motor activity counts (mean ± SD)				
Testing day	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Males				
Day 14	63.5 ± 81.7	49.3 ± 97.4 (↓22%)	17.5 ± 24.3 (↓72%)	18.8 ± 23.7 (↓70%)
Day 18	101.7 ± 120.6	78.1 ± 93.6	64.8 ± 67.0	110.9 ± 109.0
Day 22	273.7 ± 160.6	222.5 ± 118.5	220.1 ± 181.1	291.5 ± 199.6
Day 60	470.5 ± 124.5	440.9 ± 156.7	442.9 ± 211.6	451.9 ± 108.0
Females				
Day 14	87.4 ± 98.2	81.4 ± 114.7	30.2 ± 57.6 (↓65%)	48.2 ± 63.2 (↓45%)
Day 18	192.8 ± 187.3	127.2 ± 116.8	77.8 ± 127.3 (↓60%)	127.6 ± 162.9 (↓34%)
Day 22	339.0 ± 223.1	263.4 ± 85.0	299.3 ± 214.5	237.6 ± 126.4 (↓30%)
Day 60	509.1 ± 140.3	547.1 ± 82.0	527.4 ± 124.3	511.4 ± 112.1

Data taken from Table 22, pp. 151-158, MRID 45539801.

Motor activity was decreased in PND 14 males by 22%, 72% and 70% at the low, mid, and high dose groups, respectively, when compared to controls. Individual animal data showed that the number of males considered moving appropriately for their age (defined as having at least 20 movements in any one 5-minute block) were 6/13 (46%), 9/13 (69%), 8/10 (80%), and 9/10 (90%) for control, low, mid, and high dose groups, respectively. Only three more animals did not move appropriately in the low dose compared to controls. Therefore, the mean decrease of 22% at the low dose and the three animals that had minimal movement (69-46% = 23% more than controls) were not considered to be toxicologically significant and as an adverse effect of the chemical at the low dose.

c. **Auditory startle reflex :** Data for startle amplitude and latency are given in Tables 9 and 10, respectively. No biologically-significant effects on startle response amplitude or peak latency were observed at any dose for either sex. The significant ($p<0.05$) differences from control were sporadic and not dose-related and are considered incidental to treatment. Habituation was evident in all groups of males and females on both test days as a decrease in response amplitude over the test session.

TABLE 9: Auditory startle peak amplitude (v)					
Day	**Repetition**	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Males (n = 10-13)					
23	1-10	214.0 ± 69.1	206.7 ± 69.3	256.3 ± 72.8	216.0 ± 75.5
	11-20	187.0 ± 66.6	182.9 ± 63.1	169.8 ± 56.6	196.8 ± 70.0
	21-30	162.5 ± 61.5	178.1 ± 53.8	166.6 ± 60.0	180.1 ± 77.2
	31-40	163.1 ± 46.7	152.9 ± 54.1	145.8 ± 57.5	163.6 ± 84.1
	41-50	163.5 ± 47.7	150.7* ± 47.5	133.7** ± 45.4	157.5 ± 80.0
61	1-10	817.1 ± 318.4	712.8 ± 437.1	746.6 ± 432.9	649.9 ± 331.8
	11-20	693.5 ± 410.5	587.0 ± 285.4	666.7 ± 244.2	518.1 ± 420.8
	21-30	586.9 ± 311.4	517.3 ± 334.5	577.5 ± 309.1	491.4 ± 454.3
	31-40	521.6 ± 205.3	477.9 ± 229.5	463.8 ± 289.3	479.2 ± 385.2
	41-50	526.5 ± 258.0	404.3 ± 270.0	441.0 ± 255.6	340.7 ± 238.9
Females (n = 9-13)					
23	1-10	238.7 ± 95.8	224.8 ± 82.9	231.1 ± 65.5	216.1 ± 43.7
	11-20	184.1 ± 71.2	178.5 ± 62.0	205.6 ± 47.7	191.6 ± 38.9
	21-30	158.6 ± 69.8	157.7 ± 61.2	193.9 ± 49.9	162.7 ± 50.8
	31-40	151.4 ± 59.5	159.6 ± 48.1	171.1 ± 68.1	165.1 ± 46.1
	41-50	156.6 ± 67.1	153.6 ± 56.3	151.9 ± 38.8	141.8 ± 50.6
61	1-10	515.8 ± 261.6	570.0 ± 236.4	525.4 ± 180.3	482.3 ± 231.4
	11-20	476.2 ± 316.5	523.0 ± 274.6	570.6 ± 203.6	477.6 ± 272.3
	21-30	466.5 ± 266.6	505.0 ± 268.6	432.3 ± 228.2	431.9 ± 222.9
	31-40	442.3 ± 273.6	385.3 ± 221.6	417.7 ± 212.8	383.4 ± 289.0
	41-50	410.5 ± 264.9	334.6 ± 171.7	490.0 ± 173.5	397.0 ± 234.5

Data taken from Table 23, pp. 159-162, MRID 45539801.
Significantly different from control: *p ≤ 0.05; **p ≤0.01.

TABLE 10: Auditory startle time to maximum amplitude (ms)					
Day	Repetition	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
Males (n = 10-13)					
23	1-10	25.3 ± 5.3	23.2 ± 3.3	27.5 ± 5.6	23.8 ± 4.1
	11-20	20.4 ± 1.9	21.4 ± 2.9	22.1* ± 1.6	21.6 ± 2.8
	21-30	21.6 ± 3.0	20.7 ± 2.4	21.7 ± 1.6	20.5 ± 2.6
	31-40	20.2 ± 2.1	21.5 ± 3.4	22.0 ± 2.7	21.5 ± 3.4
	41-50	19.9 ± 2.0	21.3 ± 3.2	22.0* ± 2.7	21.0 ± 2.8
61	1-10	24.4 ± 3.9	27.1 ± 6.9	28.5 ± 9.7	27.7 ± 10.2
	11-20	25.3 ± 5.5	26.9 ± 6.0	25.4 ± 3.0	28.8 ± 11.0
	21-30	27.4 ± 4.2	26.7 ± 6.8	27.5 ± 7.9	29.7 ± 6.6
	31-40	25.3 ± 4.0	26.8 ± 4.4	26.4 ± 6.7	28.1 ± 7.0
	41-50	26.3 ± 4.5	29.2 ± 9.5	29.8 ± 7.9	31.5 ± 8.1
Females (n = 9-13)					
23	1-10	25.3 ± 4.4	24.2 ± 3.3	22.7 ± 3.8	22.7 ± 2.8
	11-20	21.7 ± 2.0	20.4 ± 2.2	20.1 ± 1.9	23.8 ± 3.0
	21-30	21.8 ± 3.5	19.8 ± 1.6	21.5 ± 4.5	22.1 ± 3.0
	31-40	22.7 ± 5.0	20.6 ± 2.9	25.1 ± 8.8	22.1 ± 3.2
	41-50	20.5 ± 3.1	21.1* ± 3.8	21.3 ± 2.7	22.2 ± 2.6
61	1-10	26.1 ± 6.1	24.7 ± 4.2	26.3 ± 3.5	25.7 ± 3.0
	11-20	24.7 ± 5.3	25.1 ± 6.3	24.8 ± 2.4	25.3 ± 4.8
	21-30	27.0 ± 6.8	26.2 ± 7.2	26.3 ± 6.2	25.3 ± 3.3
	31-40	26.7 ± 8.9	27.1 ± 10.7	25.8 ± 2.6	26.6 ± 5.2
	41-50	26.8 ± 7.9	26.0 ± 7.6	25.9 ± 4.7	26.0 ± 4.1

Data taken from Table 24, pp. 163-166, MRID 45539801.
Significantly different from control: *$p \leq 0.05$.

d. Learning and memory testing:

Passive avoidance: Tests for passive avoidance were not conducted.

Water maze: Overall water maze data are presented as follows: Table 11 shows the percentage of successful trials completed in less than the cut-off time in seconds for each testing interval. Only about 50% of the control rats successfully completed the task within 10 seconds on PND 21, while > 80% fo the control rats were successful within 10 seconds on PND 24. Thus, learning and memory of the task was achieved.

In contrast, comparison of the day 59 and day 62 successful trials indicated that both the learning (day 59) and memory (day 62) interval response curves were similar, so there was no memory demonstrated in controls between the three days. The females may have actually had a lower response rate in the memory phase than in the learning phase since only a cumulative success rate of 72.7% was achieved for the day 62 memory phase controls vs a cumulative success rate of 93.6% for the day 59 learning phase.

Table 11 also shows that the high dose group males on PND 62 were consistently lower in cumulative successes at each time interval. In particular, the high dose group males were 95%, 71%, 43%, 39%, 24%, 20% and 11% lower than controls on PND 62 at 3, 4, 5, 6, 7, 8 and 9 seconds and all of these differences were statistically significantly different. At 10 seconds, the difference remained 8% lower but the difference was not statistically significant. This data demonstrates that the high dose males responded slower to finding the escape ladder than the other groups.

Table 12 gives the mean percentage for successful trials out of all trials when a successful trial is defined as 1.5× the straight channel swim speed. The high dose group males had fewer successful (29%) trials than controls on PNDs 24, 59 and 62.

In Table 13, the mean time per trial is given for each testing interval. The high dose group males were statistically significantly slower than controls for trials 1 (122%), 2 (65%), 3 (78%), 5 (42%) and 6 (65%) with trials 1, 5, and 6 reaching statistical significance. However, males and females failed to learn..

TABLE 11: Water maze percentage of successful trials[a]								
Cut-off	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
	Males				Females			
Day 21 - Learning phase								
3 sec	1.3 ± 4.6	1.4 ± 4.8	4.5 ± 10.8	0.0 ± 0.0	2.6 ± 9.2	2.6 ± 9.2	1.5 ± 5.0	1.7 ± 5.3
4 sec	10.3 ± 17.4	11.1 ± 19.2	6.1 ± 11.2	3.7 ± 7.3	7.7 ± 16.1	10.3 ± 18.7	6.1 ± 11.2	20.0 ± 20.5
5 sec	16.7 ± 19.2	15.3 ± 19.4	13.6 ± 14.6	7.4 ± 12.1	17.9 ± 20.9	19.2 ± 23.4	12.1 ± 15.1	26.7 ± 23.8
6 sec	25.6 ± 18.8	25.0 ± 24.1	27.3 ± 18.7	18.5 ± 17.6	30.8 ± 27.9	26.9 ± 22.1	19.7 ± 16.4	31.7 ± 18.3
7 sec	34.6 ± 17.3	37.5 ± 24.7	34.8 ± 20.4	29.6 ± 28.6	37.2 ± 29.8	33.3 ± 18.0	24.2 ± 18.8	40.0 ± 22.5
8 sec	37.2 ± 19.4	40.3 ± 26.1	39.4 ± 18.7	31.5 ± 26.9	39.7 ± 27.7	38.5 ± 19.7	27.3 ± 18.7	56.7 ± 22.5
9 sec	47.4 ± 22.4	43.1 ± 25.1	45.5 ± 13.1	35.2 ± 25.6	47.4 ± 27.1	44.9 ± 17.2	33.3 ± 25.8	61.7 ± 19.3
10 sec	50.0 ± 22.6	50.0 ± 27.5	48.5 ± 13.9	37.0 ± 24.7	51.3 ± 26.8	50.0 ± 21.5	39.4 ± 25.0	66.7 ± 15.7
Day 24 - Memory phase								
3 sec	7.7 ± 12.9	6.9 ± 11.1	4.5 ± 10.8	5.6 ± 8.3	12.8 ± 13.9	15.4 ± 18.6	16.7 ± 23.6	6.7 ± 14.1
4 sec	30.8 ± 28.7	18.1 ± 16.6	21.2 ± 28.0	22.2 ± 26.4	38.5 ± 14.2	30.8 ± 20.2	31.8 ± 22.9	26.7 ± 23.8
5 sec	47.4 ± 26.2	34.7 ± 27.0	34.8 ± 27.3	29.6 ± 27.4	48.7 ± 17.3	43.6 ± 22.1	45.5 ± 27.0	41.7 ± 28.6
6 sec	57.7 ± 24.2	52.8 ± 27.4	50.0 ± 29.8	51.9 ± 26.9	59.0 ± 17.5	57.7 ± 25.1	62.1 ± 21.2	60.7 ± 26.3
7 sec	69.2 ± 24.4	65.3 ± 16.6	60.6 ± 31.9	55.6 ± 26.4	65.4 ± 22.0	66.7 ± 24.5	65.2 ± 21.7	66.7 ± 19.2
8 sec	76.9 ± 23.1	76.4 ± 15.0	69.7 ± 32.3	59.3 ± 30.2	71.8 ± 19.7	73.1 ± 21.0	69.7 ± 22.1	78.3 ± 22.3
9 sec	82.1 ± 18.6	76.4 ± 15.0	72.7 ± 30.1	66.7 ± 30.0	82.1 ± 17.3	78.2 ± 15.8	77.3 ± 11.2	85.0 ± 14.6
10 sec	82.1 ± 18.6	83.3 ± 10.1	78.8 ± 22.5	72.2 ± 32.3	82.1 ± 17.3	83.3 ± 13.6	80.3 ± 12.5	86.7 ± 13.1
Day 59 - Learning phase								
3 sec	34.6 ± 30.0	22.2 ± 28.7	16.7* ±25.8	27.8 ± 22.0	35.9 ± 27.1	26.9 ± 25.0	30.3 ± 30.6	28.3 ± 27.3
4 sec	59.0 ± 29.4	50.0 ± 26.6	40.9** ± 25.1	59.3 ± 16.9	57.7 ± 21.1	50.0 ± 22.6	42.4 ± 26.2	53.3 ± 23.3
5 sec	67.9 ± 29.2	56.9 ± 21.9	56.1* ±20.1	70.4 ± 13.9	66.7 ± 16.7	61.5 ± 19.7	57.6 ± 20.2	61.7 ± 26.1
6 sec	79.5 ± 18.2	68.1 ± 21.9	69.7 ± 23.4	75.9 ± 16.9	75.6 ± 12.9	70.5 ± 18.2	63.6 ± 20.8	76.7 ± 14.1

TABLE 11: Water maze percentage of successful trials[a]								
Cut-off	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
7 sec	84.6 ± 12.7	72.2 ± 19.2	80.3 ± 19.5	81.5 ± 15.5	82.1 ± 14.4	80.8 ± 15.0	69.7 ± 20.8	80.0 ± 13.1
8 sec	89.7 ± 10.8	81.9 ± 15.0	83.3 ± 16.7	85.2 ± 13.0	87.2 ± 13.9	87.2 ± 15.4	74.2 ± 20.2	86.7 ± 10.5
9 sec	92.3 ± 8.6	81.9* ±15.0	89.4 ± 13.5	88.9 ± 14.4	88.5 ± 12.5	91.0 ± 16.1	80.3 ± 20.8	88.3 ± 11.2
10 sec	93.6 ± 8.4	84.7 ± 15.0	89.4 ± 13.5	92.6 ± 12.1	93.6 ± 10.8	91.0 ± 16.1	86.4 ± 19.5	88.3 ± 11.2
Day 62 - Memory phase								
3 sec	36.4 ±20.8	33.3* ±28.3	40.0* ±30.6	1.9* ± 5.6 (↓95%)	25.8 ± 27.2	15.2 ± 18.9	37.0 ± 32.0	30.0 ± 18.9
4 sec	57.6 ± 26.2	46.7 ± 28.1	61.7 ± 30.5	16.7** ±16.7 (↓71%)	43.9 ± 28.2	30.3 ± 28.7	53.7 ± 29.8	41.7 ± 21.2
5 sec	68.2 ± 17.4	53.3 ± 24.6	70.0 ± 30.2	38.9* ±20.4 (↓43%)	45.5 ± 27.0	36.4 ± 25.6	57.4 ± 32.4	43.3 ± 21.1
6 sec	78.8 ± 18.4	68.3 ± 14.6	80.0 ± 21.9	48.1* ± 17.6 (↓39%)	50.0 ± 28.9	47.0 ± 27.7	61.1 ± 32.3	56.7 ± 22.5
7 sec	83.3 ± 16.7	81.7 ± 20.0	85.0 ± 12.3	63.0* ±13.9 (↓24%)	56.1 ± 28.2	51.5 ± 25.2	70.4 ± 30.9	63.3 ± 20.5
8 sec	87.9 ± 15.1	86.7 ± 17.2	93.3 ± 8.6	70.4** ± 11.1 (↓20%)	65.2 ± 26.3	57.6 ± 26.2	74.1 ± 27.8	66.7 ± 19.2
9 sec	89.4 ± 11.2	90.0 ± 11.7	95.0 ± 8.1	79.6* ±13.9 (↓11%)	69.7 ± 28.7	62.1 ± 23.7	77.8 ± 28.9	71.7 ± 22.3
10 sec	90.9 ± 8.7	93.3 ± 11.7	98.3 ± 5.3	83.3 ± 14.4 (↓8%)	72.7 ± 26.1	63.6 ± 22.1	81.5 ± 28.2	73.3 ± 19.6

Data taken from Table 19, pp. 134-141, MRID 45539801.
[a]A successful trial is one completed in less than the cut-off time.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

TABLE 12. Mean percentage of successful trials at 1.5× straight channel swim time				
Interval (phase)	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Males				
Day 21 (learning)	38.5 ± 34.3	33.3 ± 36.2	33.3 ± 28.9	37.0 ± 36.1
Day 24 (memory)	55.1 ± 36.9	68.1 ± 31.3	53.0 ± 44.6	46.3 ± 37.1
Day 59 (learning)	66.7 ± 28.1	43.1* ± 28.8	50.0 ± 31.6	57.4 ± 29.0
Day 62 (memory)	56.1 ± 21.4	58.3 ± 30.7	63.3 ± 29.2	38.9 ± 20.4
Females				
Day 21 (learning)	24.4 ± 27.7	34.6* ± 26.8	15.2 ± 17.4	30.0* ± 27.0
Day 24 (memory)	57.7 ± 35.1	51.3 ± 29.2	43.9 ± 38.2	43.3 ± 34.4
Day 59 (learning)	59.0 ± 23.2	52.6 ± 22.4	42.4 ± 36.0	55.0 ± 23.6
Day 62 (memory)	45.5 ± 30.8	40.9 ± 20.2	51.9 ± 32.7	45.0 ± 20.9

Data taken from Table 20, p. 142, MRID 45539801.
Significantly different from control: *$p \leq 0.05$.

TABLE 13: Water maze mean time per trial (sec)								
Trial	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day	0 mg/kg/day	10 mg/kg/day	25 mg/kg/day	100 mg/kg/day
	Males				Females			
Day 21 - Learning phase								
Trial 1	13.74± 5.90	13.62 ± 5.45	12.67 ± 5.93	14.76 ± 6.52	13.73 ± 4.87	13.79 ± 4.63	14.35 ± 7.66	13.66 ± 6.76
Trial 2	9.37 ± 5.12	13.29 ± 8.18	9.05 ± 5.36	17.89* ± 9.56	8.94 ± 4.43	15.39* ± 9.05	13.11 ± 7.53	7.41 ± 6.01
Trial 3	10.27 ± 4.82	13.94 ± 8.95	13.06±8.9 4	13.95±9.9 2	11.23 ± 7.20	9.99 ± 5.18	14.48 ± 8.79	10.52 ± 5.45
Trial 4	10.94 ± 4.86	11.45±7.4 1	14.88±8.6 6	17.06* ± 8.63	13.02 ± 9.11	9.54 ± 8.38	14.48 ± 8.80	9.51 ± 4.49
Trial 5	9.08 ± 5.92	10.13±6.4 7	11.42±7.4 2	9.53 ± 6.57	9.06 ± 5.57	7.48 ± 4.04	11.21 ± 6.80	8.82 ± 6.54
Trial 6	12.63 ± 10.53	8.78 ± 6.81	8.58 ± 3.36	11.02 ± 8.18	10.34 ± 7.87	11.64 ± 6.55	12.67 ± 9.80	7.75 ± 6.25
Day 24 - Memory phase								
Trial 1	8.30 ± 3.68	8.93 ± 3.46	10.75±7.9 8	8.76 ± 3.53	8.61 ± 4.47	9.87 ± 2.79	12.91 ± 8.16	7.71 ± 5.92
Trial 2	6.67 ± 5.51	8.38 ± 5.13	7.90 ± 3.66	7.59 ± 5.03	5.35 ± 2.91	5.65 ± 3.59	8.71 ± 7.68	9.61 ± 7.45
Trial 3	5.25 ± 1.82	5.54 ± 2.03	7.05 ± 6.52	6.74 ± 3.84	4.26 ± 1.49	4.72 ± 1.86	4.88 ± 2.06	4.82 ± 0.91
Trial 4	6.71 ± 3.78	6.03 ± 2.84	7.74 ± 4.22	8.60 ± 5.41	7.01 ± 7.55	5.90 ± 3.04	5.18 ± 4.18	6.57 ± 3.23
Trial 5	6.13 ± 3.27	6.38 ± 2.81	6.92 ± 4.52	7.23 ± 4.29	7.57 ± 3.61	6.62 ± 4.60	6.99 ± 5.09	6.14 ± 1.80
Trial 6	6.30 ± 6.07	5.80 ± 3.23	6.67 ± 4.32	10.39 ± 9.71	7.54 ± 5.37	5.74 ± 3.33	5.64 ± 2.26	4.95 ± 2.4
Day 59 - Learning phase								
Trial 1	8.15 ± 4.18	9.44 ± 3.85	9.32 ± 4.44	7.83 ± 5.48	8.35 ± 2.59	9.70 ± 6.62	10.34 ± 5.74	11.25 ±10.02
Trial 2	4.16 ± 2.10	6.73 ± 4.34	4.31 ± 1.68	6.68* ± 3.43	4.73 ± 2.38	7.15 ± 7.99	8.81 ± 10.15	4.37 ± 2.76
Trial 3	3.82 ± 2.44	5.53 ± 5.69	5.51 ± 3.15	4.70 ± 3.98	3.64 ± 1.71	4.33 ± 3.42	5.65 ± 3.47	5.83 ± 7.72
Trial 4	4.33 ± 2.04	3.60 ± 1.46	4.54 ± 2.31	3.46 ± 0.74	3.88 ± 2.44	3.73 ± 1.79	6.87 ± 6.78	4.24 ± 2.92
Trial 5	3.76 ± 1.74	4.42 ± 3.66	3.87 ± 1.05	3.30 ± 0.57	3.29 ± 1.14	4.44 ± 1.88	3.67 ± 1.13	4.03 ± 1.95
Trial 6	3.42 ± 1.29	6.88 ± 8.12	5.77 ± 5.38	4.32 ± 2.10	3.98 ± 1.96	4.19 ± 1.81	4.46 ± 2.69	5.10 ± 3.02
Day 62 - Memory phase								
Trial 1	3.72 ± 1.66	4.48 ± 1.89	4.82 ± 2.41	8.25** ± 4.24 (1122%)	9.00 ± 4.17	5.64 ± 3.59	7.76 ± 4.53	5.24* ± 3.77
Trial 2	4.24 ± 3.05	4.79 ± 2.82	3.87 ± 1.56	6.98 ± 2.98	5.11 ± 5.22	7.89 ± 7.27	5.35 ± 4.80	4.74 ± 2.74

TABLE 13: Water maze mean time per trial (sec)								
Trial	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Trial 3	5.56 ± 3.64	6.16 ± 4.35	4.19 ± 2.43	9.88 ± 8.96 (178%)	8.22 ± 8.79	8.92 ± 6.96	9.12 ± 9.31	6.47 ± 4.88
Trial 4	6.84 ± 5.48	4.46 ± 2.67	4.20 ± 2.22	5.80 ± 1.77	7.93 ± 6.50	11.69±7.9 7	6.12 ± 7.95	9.91 ± 7.28
Trial 5	3.84 ± 1.35	4.29 ± 2.29	4.64 ± 2.32	5.44* ±1.30 (142%)	9.40 ± 8.02	11.95±9.7 2	5.23 ± 4.18	8.22 ± 6.95
Trial 6	4.37 ± 1.97	5.89 ± 2.90	3.91 ± 2.35	7.19* ±3.76 (165%)	7.80 ± 5.02	9.68 ± 6.49	5.11 ± 3.47	10.23±8.5 1

Data taken from Table 21, pp. 143-150, MRID 45539801.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

e. **Ophthalmology:** Eyes were not specifically examined other than for obvious effects when clinical signs and FOB was assessed..

5. Postmortem results:

a. **Brain weights:** Mean brain weight data are given in Table 14. Absolute and relative brain weights of male and female offspring were similar between the treated and control groups on PND 12 and at study termination.

TABLE 14. Mean (±SD) brain weight data for offspring				
Parameter	**0 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**
Males				
Day 12 (n = 10)				
Terminal body weight (g)	21.7 ± 4.0	21.9 ± 2.0	20.9 ± 3.9	19.2 ± 3.6
Brain weight (g)	1.09 ± 0.16	1.07 ± 0.08	1.05 ± 0.15	1.01 ± 0.13
Brain-to-body weight ratio (%)	5.12 ± 0.81	4.95 ± 0.78	5.12 ± 0.78	5.42 ± 1.33
Termination (n = 20)				
Terminal body weight (g)	342.0 ± 21.8	337.2 ± 21.6	339.7 ± 27.1	331.2 ± 30.0
Brain weight (g)	1.97 ± 0.12	1.98 ± 0.13	1.96 ± 0.16	1.95 ± 0.09
Brain-to-body weight ratio (%)	0.58 ± 0.05	0.59 ± 0.05	0.58 ± 0.06	0.59 ± 0.04
Females				
Day 12 (n = 9-10)				
Terminal body weight (g)	21.6 ± 1.7	20.9 ± 2.0	22.2 ± 2.5	19.2 ± 2.8
Brain weight (g)	1.11 ± 0.13	1.08 ± 0.19	1.11 ± 0.12	1.06 ± 0.16
Brain-to-body weight ratio (%)	5.14 ± 0.49	5.19 ± 0.75	5.03 ± 0.71	5.58 ± 1.00
Termination (n = 20)				
Terminal body weight (g)	205.4 ± 23.8	213.0 ± 18.8	207.4 ± 12.8	208.2 ± 16.7
Brain weight (g)	1.83 ± 0.11	1.83 ± 0.09	1.83 ± 0.14	1.83 ± 0.08
Brain-to-body weight ratio (%)	0.90 ± 0.09	0.86 ± 0.06	0.88 ± 0.06	0.88 ± 0.05

Data taken from Table 28, pp. 172-173, MRID 45539801.

C. NEUROPATHOLOGY

1. **Macroscopic examination:** No treatment-related gross lesions were reported at postnatal day 12 or at study termination.

2. **Microscopic examination:** No treatment-related microscopic lesions were noted on postnatal days 12 or 63.

3. **Brain morphometry:** Brain measurement data for days 12 and 63 are given in Tables 15 and 16, respectively. No treatment-related morphometric effects were observed in any animals on either day; several statistical differences in individual measurements were not consistent in location, over time, or between sexes.

TABLE 15: Offspring brain measurements on day 12				
Measurement	**0 mg/kg/day**	**100 mg/kg/day**	**0 mg/kg/day**	**100 mg/kg/day**
	Males (n = 6)		**Females (n = 6)**	
Cerebellum				
Height (mm)	3.328 ± 0.317	3.380 ± 0.446	3.650 ± 0.465	3.386 ± 0.420
Length (mm)	3.870 ± 0.583	4.000 ± 0.744	3.967 ± 0.158	3.892 ± 0.316
Preculminate Fissure				
Outer granular layer (µm)	38.05 ± 10.12	37.34 ± 6.16	37.49 ± 6.57	36.74 ± 4.32
Inner granular layer (µm)	130.1 ± 21.0	131.2 ± 18.8	138.6 ± 16.6	147.6 ± 11.5
Molecular layer (µm)	66.9 ± 14.0	66.3 ± 11.7	71.1 ± 4.0	66.2 ± 8.7
Prepyramidal Fissure				
Outer granular layer (µm)	44.128 ± 5.783	44.282 ± 5.329	45.105 ± 3.756	40.542 ± 2.983
Inner granular layer (µm)	118.9 ± 8.8	129.3 ± 30.6	142.0 ± 14.8	111.9** ± 13.8
Molecular layer (µm)	49.9 ± 11.3	50.5 ± 8.6	60.4 ± 7.0	50.9 ± 11.9
Level 5				
Dorsal cortex (mm)	0.893 ± 0.063	0.895 ± 0.057	0.958 ± 0.084	0.880 ± 0.170
Pyriform cortex (mm)	0.890 ± 0.130	0.826 ± 0.077	0.842 ± 0.140	0.811 ± 0.127
Thalamus width (mm)	5.405 ± 0.567	6.093 ± 0.560	5.995 ± 0.071	5.677* ± 0.243
Hippocampus - width dentate gyrus (mm)	0.586 ± 0.068	0.583 ± 0.056	0.668 ± 0.097	0.582 ± 0.056
Hippocampus width overall (mm)	1.133 ± 0.111	1.165 ± 0.075	1.303 ± 0.078	1.150* ± 0.106
Level 4				
Dorsal cortex (mm)	0.979 ± 0.095	0.980 ± 0.050	1.023 ± 0.114	0.959 ± 0.089
Pyriform cortex (mm)	0.928 ± 0.109	0.925 ± 0.107	1.024 ± 0.052	0.928* ± 0.066
Corpus callosum (mm)	0.495 ± 0.146	0.520 ± 0.070	0.515 ± 0.072	0.595 ± 0.163
Thalamus height (mm)	4.342 ± 0.256	4.245 ± 0.335	4.207 ± 0.364	4.363 ± 0.379
Thalamus width (mm)	6.758 ± 0.394	6.702 ± 0.696	6.850 ± 0.592	7.088 ± 0.782
Thalamus/cortex width (mm)	11.55 ± 0.51	11.46 ± 0.73	11.62 ± 0.44	11.85 ± 1.09
Hippocampus - length from midline (mm)	3.689 ± 0.294	3.710 ± 0.370	3.366 ± 0.436	3.783 ± 0.282
Hippocampus - width dentate gyrus (mm)	0.357 ± 0.062	0.349 ± 0.041	0.388 ± 0.028	0.374 ± 0.036
Hippocampus - length dentate gyrus (mm)	1.233 ± 0.163	1.255 ± 0.153	1.336 ± 0.148	1.210 ± 0.124
Level 3				
Dorsal cortex 1 (mm)	1.113 ± 0.066	1.235* ± 0.116	1.188 ± 0.069	1.116 ± 0.098
Dorsal cortex 2 (mm)	1.110 ± 0.133	1.233 ± 0.081	1.228 ± 0.120	1.224 ± 0.116
Pyriform cortex (mm)	0.997 ± 0.075	1.058 ± 0.107	1.163 ± 0.045	1.078* ± 0.075
Hippocampus - length from midline (mm)	2.738 ± 0.377	2.513 ± 0.219	2.693 ± 0.181	2.558 ± 0.258
Level 2				
Height (mm)	5.688 ± 0.655	5.313 ± 0.356	5.210 ± 0.383	5.367 ± 0.190
Width (mm)	4.354 ± 0.352	4.159 ± 1.777	4.118 ± 0.381	3.994 ± 0.449

Data taken from Table 29, pp. 174-201, MRID 45539801.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 16: Offspring brain measurements on day 63				
Measurement	**0 mg/kg/day**	**100 mg/kg/day**	**0 mg/kg/day**	**100 mg/kg/day**
	Males (n = 6)		**Females (n = 6)**	
Cerebellum				
Height (mm)	4.735 ± 0.639	5.083 ± 0.266	4.652 ± 0.327	4.748 ± 0.394
Length (mm)	6.586 ± 0.141	6.700 ± 0.194	6.515 ± 0.326	6.603 ± 0.390
Preculminate Fissure				
Inner granular layer (µm)	167.8 ± 28.4	160.1 ± 22.4	150.6 ± 34.7	164.1 ± 21.0
Molecular layer (µm)	193.0 ± 30.4	219.6 ± 9.4	193.8 ± 17.2	222.8 ± 41.9
Prepyramidal Fissure				
Inner granular layer (µm)	146.1 ± 24.0	172.9 ± 23.5	149.9 ± 26.8	169.2 ± 50.3
Molecular layer (µm)	187.5 ± 13.6	193.9 ± 9.0	191.4 ± 25.2	187.1 ± 19.3
Level 5				
Dorsal cortex (mm)	1.169 ± 0.078	1.225 ± 0.072	1.144 ± 0.091	1.192 ± 0.092
Pyriform cortex (mm)	1.022 ± 0.066	0.980 ± 0.100	1.004 ± 0.122	0.941 ± 0.089
Thalamus width (mm)	7.332 ± 0.229	7.173 ± 0.472	7.002 ± 0.325	6.982 ± 0.631
Hippocampus - width dentate gyrus (mm)	0.603 ± 0.037	0.645 ± 0.063	0.638 ± 0.070	0.632 ± 0.102
Hippocampus width overall (mm)	1.343 ± 0.075	1.331 ± 0.106	1.353 ± 0.104	1.337 ± 0.141
Level 4				
Dorsal cortex (mm)	1.398 ± 0.053	1.206* ± 0.127	1.211 ± 0.046	1.284 ± 0.069
Pyriform cortex (mm)	1.085 ± 0.094	0.981 ± 0.125	1.077 ± 0.089	1.016 ± 0.030
Corpus callosum (mm)	0.420 ± 0.053	0.43 ± 0.086	0.417 ± 0.088	0.467 ± 0.051
Thalamus height (mm)	4.702 ± 0.457	4.550 ± 0.369	4.872 ± 0.221	4.670 ± 0.148
Thalamus width (mm)	7.340 ± 0.423	7.702 ± 0.434	7.598 ± 0.305	7.670 ± 0.335
Thalamus/cortex width (mm)	12.88 ± 0.42	13.02 ± 0.75	12.98 ± 0.40	12.77 ± 0.33
Hippocampus - length from midline (mm)	3.270 ± 0.503	3.586 ± 0.442	3.620 ± 0.227	3.557 ± 0.226
Hippocampus - width dentate gyrus (mm)	0.511 ± 0.037	0.507 ± 0.073	0.502 ± 0.020	0.508 ± 0.027
Hippocampus - length dentate gyrus (mm)	1.338 ± 0.213	1.602* ± 0.131	1.520 ± 0.117	1.596 ± 0.195
Level 3				
Dorsal cortex 1 (mm)	1.247 ± 0.156	1.292 ± 0.103	1.308 ± 0.073	1.396 ± 0.096
Dorsal cortex 2 (mm)	1.492 ± 0.081	1.455 ± 0.102	1.473 ± 0.098	1.514 ± 0.108
Pyriform cortex (mm)	1.151 ± 0.056	1.070 ± 0.135	1.210 ± 0.140	1.108 ± 0.049
Hippocampus - length from midline (mm)	2.206 ± 0.614	2.646 ± 0.517	2.978 ± 0.405	2.783 ± 0.465
Level 2				
Height (mm)	5.908 ± 0.382	6.053 ± 0.539	5.855 ± 0.257	5.988 ± 0.503
Width (mm)	4.420 ± 0.348	4.581 ± 0.291	4.434 ± 0.210	4.405 ± 0.275

Data taken from Table 29, pp. 202-227, MRID 45539801.
Significantly different from control: *$p \leq 0.05$.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** Maternal toxicity was evident at 100 mg/kg/day as salivation and reduced body weight and food consumption during gestation. The low incidence of salivation in dams administered 25 mg/kg/day was considered to be of no toxicological significance.

The investigators concluded that the overall no effect level is 25 mg/kg/day for developmental neurotoxicity. The high dose of 100 mg/kg/day was associated with reduced pup survival, transient (while the dams were exposed) decrease in body weight and a difference in performance of the males in the water maze. There were no morphologic changes in neural tissues in offspring. The study author did not regard the reduced motor activity as an effect of treatment.

The study author also concluded (page 31 of the study report) that "no treatment related effects on the performance in the water maze of males or females given 10 or 25 mg/kg/day of females given 100 mg/kg/day were evident from evaluation of the data in 3 different ways. However, an effect of treatment with 100 mg/kg/day on the performance of the males in the water maze cannot be dismissed."

The investigators did not conclude that there was an effect of treatment on motor activity in either sex on days 14 and 18. The following is an explanation provided by the study author (page 34 of the study report).

"Generally, locomotor activity on day 14 was lower in the 25 and 100 mg/kg/day groups than in the control group, but the differences rarely achieved statistical significance. Consideration of the individual data revealed that the majority of animals in all groups showed little or no activity in the monitoring cages. Occasional animals were notably more active, particularly in the control group, and this is reflected in the larger standard deviations. For the animals in the glyphosate trimesium groups, individual values for specific time points and the overall activity were within the range of concurrent controls. Thus, the difference in locomotor activity on day 14 was considered incidental to maternal treatment with glyphosate trimesium.

B. **REVIEWER COMMENTS:** No treatment-related effects were seen in the dams on survival, clinical signs, FOB, or reproductive performances at any dose level. Treatment-related effects for maternal animals included salivation and decreased body weight and food consumption for high-dose dams. A low incidence of salivation in the mid-dose dams is related to treatment but is not regarded as sufficient to be included as an adverse effect. The body weight effects during gestation were considered transitory and also not an adverse effect and the salivation was not robust to serve as the basis for a LOAEL.

In the offspring, treatment had no adverse effects on developmental landmarks, FOB, auditory startle, learning and memory, brain weights, brain morphology or neuropathology. No treatment-related effects were seen on survival, body weight or body weight gainsof pups at the low and mid dose groups. At the high dose (100 mg/kg/day) there was a decrease (19%) in survival on Day 5 and during Days 1-5, the percentage of pup survival was lower (84.8%) when compared to controls (93%). At 100 mg/kg/day, body weight gains for the high-dose males and females were reduced approximately 10% during lactation days 1-5. Overall motor activity was decreased in males and females at the mid and high dose groups on lactation day 14. Activities were decreased to 72% and 65% for the mid-dose males and females, respectively, and to 70% and 45% for the high-dose males and females, respectively. At day 18, activities in females were reduced 60% in the mid dose group and only 34% in the high dose group.

The maternal NOAEL is greater than 100 mg/kg/day; the highest dose tested. The maternal LOAEL was not established.

The offspring LOAEL is 25 mg/kg/day, based on dose-dependent decreases in motor activity in males and females on PND 14. The offspring NOAEL is 10 mg/kg/day.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of learning and memory in the offspring and the pending review of the of positive control data.

C. DEFICIENCIES

Tests for passive avoidance were not conducted.

APPENDIX: DOSE SELECTION RATIONALE

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 128501 **DP BARCODE:** D285565
SUBMISSION NO.: S621853

TEST MATERIAL (PURITY): Technical Grade Glyphosate trimesium (57.4%)

SYNONYMS:

CITATION: Moxon, M.E. (1999) Glyphosate trimesium: Second preliminary developmental neurotoxicity study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Laboratory study number RR0826; August 27, 1999. MRID 45539802. Unpublished

Moxon, M.E. (1999) Glyphosate trimesium: Preliminary developmental neurotoxicity study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Laboratory study number RR0817; August 27, 1999. MRID 45539803. Unpublished

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, PO Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In two developmental neurotoxicity range-finding studies (1999, MRID 45539802, 45539803), glyphosate trimesium (57.4% a.i., Lot # P11) was administered to 10 female Alpk:AP$_f$SD rats/group by gavage at doses of 0, 50, 100, 133, 166, or 200/25 mg/kg/day from gestation day 7 through postnatal day (PND) 11. The dose of 200 mg/kg/day was reduced to 25 mg/kg/day on GD 8 or 9 due to excessive toxicity. Maternal animals were assessed for clinical signs of toxicity, body weight changes, and food consumption. Litter data consisted of survival, sex, weight, and clinical condition of each pup up to day 12.

Maternal toxicity was evident at doses of 100 mg/kg/day and higher. At 200 mg/kg/day, one animal died after two doses and another was sacrificed moribund after one dose; necropsy of these animals was unremarkable. Marked clinical signs in this group included closed eyes, lacrimation, piloerection, altered breathing rate and depth, and subdued behavior. In the 166 mg/kg/day group three animals were found dead or sacrificed moribund on GD 18-24; fifteen dead fetuses were found *in utero* at necropsy of the animal that died on GD 24. One dam in the 133 mg/kg/day group was killed on lactation day 3 due to poor condition. Necropsy of the intercurrent deaths in the 133 and 166 mg/kg/day groups revealed distended stomach with the contents packed. Clinical signs of toxicity in the 133 and 166 mg/kg/day groups included fasciculations, hunched posture, piloerection, signs of diarrhea, and subdued behavior. Body weight loss occurred in those animals administered 200 mg/kg/day for one or two days. Adjusted body weights for the 166 mg/kg/day

group were significantly reduced beginning on GD 8 and continuing until lactation day 1. For the 133 mg/kg/day group adjusted body weights were reduced slightly throughout the study with statistical significance attained occasionally. Body weight gain was significantly reduced in the 100 mg/kg/day group on GD 8. Maternal food consumption was slightly reduced by the 100 mg/kg/day dams and significantly reduced by the higher dose groups. No effects on maternal body weight or food consumption were observed during gestation or lactation for the 25 or 50 mg/kg/day groups.

The number of pups born alive and pup survival were decreased in the 133 and 166 mg/kg/day groups such that these groups were terminated on lactation day 3 due to insufficient numbers of litters for evaluation. Pup body weights were also reduced in these groups. No effects on live litter size, pup survival, sex ratio, pup body weights, or clinical observations were found in the 25, 50, or 100 mg/kg/day groups. Therefore, doses for the main study were chosen as 0, 10, 25, and 100 mg/kg/day. Although only minimal maternal toxicity was observed with 100 mg/kg/day in the range-finding studies, the steepness of the dose-response curve precludes evaluation of higher doses.