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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

TXR# 0053106

DATE: August 24, 2005

MEMORANDUM

SUBJECT: **GLUFOSINATE AMMONIUM** - Review of Developmental Neurotoxicity Study in Rats (MRID 46455701)

PC Code: 128850
DP Barcode #: D312684

From: Robert J. Mitkus
Registration Action Branch I
Health Effects Division (7509C)

Thru: P.V. Shah, Branch Senior Scientist
Registration Action Branch I
Health Effects Division (7509C)

To: Joanne Miller
Herbicide Branch
Registration Division (7505C)

ACTION REQUESTED: The Registration Division (RD) requested Health Effects Division (HED) to perform a review of a Developmental Neurotoxicity Study in Rats (MRID 46455701) for glufosinate ammonium. This study was required as part of the conditional registration of glufosinate ammonium. The action was successfully completed, and the conclusions of the study are reported here.

I. CONCLUSIONS

The Registration Action Branch I (RAB 1) has reviewed the Developmental Neurotoxicity Study in Rats (MRID 46455701) for glufosinate ammonium. The study is classified as **Acceptable/NonGuideline** 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 pending review of the of positive control data. The submitted study satisfies the registration requirement of a Developmental Neurotoxicity

Study in Rats.

II. STUDY REVIEWED

Developmental Neurotoxicity Study - Rat; OPPTS 870.6300

CITATION: Nemec, M.D. (2004) A dietary developmental neurotoxicity study of glufosinate-ammonium in rats. WIL Research Laboratories, Inc., Ashland, Ohio. Study Number WIL-21202; August 19, 2004. MRID 46455701. Unpublished.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46455701), Glufosinate-ammonium (50.8% a.i., lot # AAKJ00053) was administered in the diet to 25 mated female Crl:CD®(SD)IGS BR rats/dose at nominal concentrations of 0, 200, 1000 and 4500 ppm from gestation day (GD) 6 through lactation day (LD) 21. Average doses to the animals were 0, 14, 69 and 292 mg/kg/day during gestation and 0, 36, 176 and 756 mg/kg/day during lactation for the 0, 200, 1000 and 4500 ppm groups, respectively. A Functional Operational Battery (FOB) was performed on 25 dams/dose on GDs 6 and 13 and on LDs 10 and 21. On postnatal day (PND) 4, litters were culled to yield five males and five females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (watermaze testing), and neuropathology at study termination (PND 72). On PND 21, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Pup physical development was evaluated at the time of body weight measurements. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No parental females died during the study. The only clinical sign was light-colored feces, which occurred primarily between GDs 8 and 13, in dams at 4500 ppm. No treatment-related clinical signs were observed in dams during the FOB. Beginning on GD 7 and continuing throughout gestation, mean body weight in the 4500 ppm group was significantly decreased (4-8%, relative to control). Mean body weight loss was observed in all treated groups at the beginning of treatment (GDs 6-7 and 6-9) but overall (GDs 6-20) weight gain was significantly decreased (27% of control value) only in the 4500 ppm group. A decrease (10% of control value) in overall weight gain at 1000 ppm was also observed. Mean food consumption during gestation was significantly decreased (8-17% of control value) in the 1000 and 4500 ppm groups. Mean body weight was slightly decreased in the 1000 and 4500 ppm groups at the beginning of lactation; however, overall (LD 1-21) mean body weight gain was increased in treated groups relative to control. Mean food consumption was significantly decreased (14% of control value) throughout lactation in the 4500 ppm group. No treatment-related effects were observed in reproductive parameters or at gross necropsy.

The maternal LOAEL for Glufosinate-ammonium in rats is 4500 ppm (292 mg/kg/day during gestation) based on decreased body weight, body weight gain, and food consumption during gestation and lactation. The maternal NOAEL is 1000 ppm (69 mg/kg/day during gestation).

No treatment-related effect on the mean number of pups born, mean live litter size or sex ratio per litter was observed. A significant treatment-related decrease in postnatal survival occurred on PNDs 0-1 and PND 0-4 in the 4500 ppm group, due mostly to total litter losses. The total number of pups found dead during the pre-weaning period was 31, 13, 17 and 60 in the control,

200, 1000 and 4500 ppm groups, respectively. Beginning on PND 4 and continuing throughout the lactation period, mean body weight was decreased in the 1000 (8-11% of control value) and 4500 ppm (13-20% of control value) male and female offspring. Mean body weight gains were decreased in the 4500 ppm male (12-35% of control value) and female (9-36% of control value) offspring and in 1000 ppm males (6-23% of control value) and females (9-23% of control value). Mean post-weaning (PNDs 28-70) body weight was decreased in males (6-9% of control value) and females (7-10% of control value) at 4500 ppm. Post-weaning body weight gain was decreased in males and females at 4500 ppm (6-9% of control value). The average onset of preputial separation in males and vaginal opening in females was not affected by treatment.

Total and ambulatory motor activity counts were increased in males and females at 1000 (1.2-2.5x control value) and 4500 ppm (1.2-2.8x control value) on most of the testing days. Statistically significant increases in several 15-minute interval motor activity counts were observed on PNDs 21 and 61 in all groups of treated males and on PND 21 in females at 1000 and 4500 ppm. No statistically significant differences between treated and control groups were observed in auditory startle response or learning and memory. Brain weight measurements and gross and microscopic necropsy findings were not affected by treatment. For the PND 72 morphometric measurements, decreased mean vertical height between the layers of pyramidal neurons in the hippocampal formation in Level 3 was observed in males at 1000 and 4500 ppm; the values in both groups were outside the historical control range. Females at 4500 ppm had significantly decreased (9% of control value) radial thickness of the cortex in Level 3, which was slightly outside the historical control range. A dose-dependent decrease in mean length of the ventral limb of the dentate hilus (Level 3) was observed in both males (9-15%) and females (12-20%) at ≥200 ppm. Statistical significance was reached in females at 200 ppm.

The offspring LOAEL for Glufosinate-ammonium in rats is 200 ppm (14 mg/kg/day during gestation) based on brain morphometric changes. The offspring NOAEL was not observed.

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 pending review of the positive control data. Additionally, glutamine synthetase measurements in the liver, kidneys, and brain of dams and pups were not measured.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509C) **Date** 8/24/05
EPA Work Assignment Manager: P.V.Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C) **Signature:** P V Shah
Date 10/24/05

TXR#: 0053106

DATA EVALUATION RECORD

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

PC CODE: 128850 **DP BARCODE:** D312684

TEST MATERIAL (PURITY): Technical Grade Glufosinate Ammonium (50.8% a.i.)

SYNONYMS: 2-amino-4-(hydroxymethylphosphinyl)butanoic acid

CITATION: Nemec, M.D. (2004) A dietary developmental neurotoxicity study of glufosinate-ammonium in rats. WIL Research Laboratories, Inc., Ashland, Ohio. Study Number WIL-21202; August 19, 2004. MRID 46455701. Unpublished.

SPONSOR: Bayer CropScience, Research Triangle Park, NC

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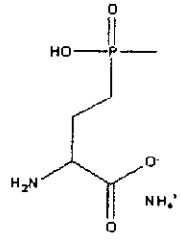
COMPLIANCE: Signed and dated Flagging, GLP, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical grade Glufosinate-Ammonium

Description:	Pale yellow liquid
Lot #:	AAKJ00053
Purity:	50.8% a.i.
Compound Stability:	Expiration date February 21, 2003
CAS # of TGAI:	77182-82-2
Structure:	

2. **Vehicle:** deionized water

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

Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Age at mating:	Males: sexually mature; females: 12 weeks
Wt. at study initiation:	Females: 226-294 g (GD 0)
Source:	Charles River Laboratories, Raleigh, NC
Housing:	Males and females in individual wire-mesh cages, except during co-habitation; individual dams and litters in plastic cages during gestation and lactation; littermates together in plastic cages until PND 28, then individually in wire-mesh cages
Diet:	PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Municipal tap drinking water, *ad libitum*
Environmental conditions:	**Temperature:** 22.1-23.2°C **Humidity:** 35.3-62.2% **Air changes:** 10/hour **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	Fourteen days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: April 15, 2002; End: June 4, 2003

2. **Study schedule:** Mated female Sprague-Dawley rats (25/dose group) were administered the test material in the diet from gestation day (GD) 6 through lactation day (LD) 21. A Functional Observational Battery (FOB) was conducted on all dams on GDs 6 and 13 and LDs 10 and 21. On postnatal day (PND) 4, litters were standardized to 10 pups; sexes were represented as equally as possible. Pups were weaned from their dam on PND 21 but were not treated with test material. Dams were sacrificed after weaning. A subset of 20 pups/sex/group was assigned to FOB (PND 4, 11, 21, 35, 45, and 60), acoustic startle

response (PND 20 and 60), locomotor activity (PND 13, 17, 21, and 61), and learning and memory testing (PND 62). From this subset, 10 pups/sex/group were selected for neuropathological, morphometric and brain weight evaluations on PND 72. A second subset of 20 pups/sex/group was selected for learning and memory (PND 22), and a third subset of 10 pups/sex/group was selected for neuropathological, morphometric and brain weight evaluations on PND 21.

3. **Mating procedure:** One resident sexually mature male and one female were co-housed. The day that a vaginal plug or sperm in a vaginal smear was observed was designated gestation day (GD) 0.

4. **Animal assignment:** Mated females were assigned to groups using a computerized randomization procedure that assigned animals based on stratification of the GD 0 body weight into a block design, as shown in Table 1.

TABLE 1. Study design

Experimental parameter	Dietary concentration (ppm)			
	0	200	1000	4500
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	25	25	25	25
FOB (GDs 6 and 13)	25	25	25	25
FOB (LDs 10 and 21)	25	25	25	25
Offspring				
	No. of offspring assigned			
Subset A - FOB (PNDs 4, 11, 21, 35, 45 and 60); Acoustic startle response (PNDs 20 and 60); Locomotor activity (PNDs 13, 17, 21 and 61); Learning and memory (PND 62).	20/sex	20/sex	20/sex	20/sex
Subset A - neuropathological, morphometric and brain weight evaluations (PND 72)	10/sex	10/sex	10/sex	10/sex
Subset B - Learning and memory (PND 22)	20/sex	20/sex	20/sex	20/sex
Subset C - Neuropathological, morphometric and brain weight evaluations (PND21)	10/sex	10/sex	10/sex	10/sex

5. **Dose selection rationale:** Dose levels were based on a preliminary range-finding study (WIL-21201) at dietary concentrations of 1000, 2000, 4000, 6000, 8000 and 10,000 ppm; results were given in summary tables in Appendix B of the study report. In maternal animals, decreased body weight gain was reported at all concentrations during gestation, with effects described as "slight" at 4,000 ppm. At 10,000 ppm, body weight loss and decreased body weight gain were observed during gestation and LD 1-4. Food consumption was reduced at ≥6,000 ppm during gestation and lactation and at 4,000 ppm during GDs 6-9 (due to decreased palatability).

In offspring, clinical observations included paleness at ≥6000 ppm, labored respiration at 8000 and 10,000 ppm and dehydration at 8000 ppm. Postnatal survival was decreased in all treated groups, including the loss of several entire litters at 10,000 ppm. Offspring body

weight and body weight gain were slightly decreased at 1000 and 2000 ppm. Body weight and body weight gain were decreased at ≥4000 ppm.

6. **Dosage administration:** Glufosinate-ammonium was administered in the diet to maternal animals on GD 6 through LD 21. After PND 21, untreated food was provided for all groups.

7. **Dosage preparation and analysis:** Appropriate amounts of the test material were weighed into tared glass vials. For dosage calculations, a correction factor of 1.97 was used to accommodate for the 50.8% purity of the test material. Specified amounts of the basal diet, one for the pre-mixture and one for the indicated batch size and appropriate dietary concentration for each group, were weighed separately. The basal diet for the pre-mixture was blended in a Hobart blender. The test material was added to the diet in the blender using a plastic pipette. The test article vials and pipettes were rinsed with deionized water to ensure the transfer of the test material to the blender. The pre-mixture was mixed for 10 minutes. For each dose group, a portion of the pre-mix was added to a V-twin shell blender, followed by a portion of the remaining basal diet. This procedure was repeated until all the diet for each group had been transferred. The formulations were then mixed for 30 minutes in the V-twin shell blender, using an intensifier bar during the first and last 5 minutes. Control diets consisted of basal diet only. Test and control diets were prepared weekly and stored at room temperature.

   On the day of the first diet preparation, duplicate samples of approximately 100 grams each were withdrawn for homogeneity and stability analyses from the top, middle, and bottom of each diet formulation. Duplicate samples for homogeneity were taken only from the middle of the control diet. One set of samples was analyzed for homogeneity. The other set (top, middle and bottom from each dose level) was combined, stored for 15 days and analyzed for stability. An approximately 100-gram sample was collected from each group throughout the study and analyzed for the concentration of the test material in the diet. When two batches were prepared for a week, both were analyzed.

Results:

Homogeneity analysis: Mean percentage of target concentrations of diet samples containing 200, 1000 and 4500 ppm were 95.3%, 109% and 108%, respectively, with relative standard deviations (RSD) of 2.3%, 3.6% and 1.8%, respectively.

Stability analysis: After 15 days at room temperature, the mean percentage of the time zero concentration was 114%, 87.1%, and 96.8% for the 200, 1000 and 4500 ppm groups, respectively.

Concentration analysis: Percentage ranges of the target concentrations were 89.5-111%, 89.9-115% and 88.6-112% for the 200, 1000 and 4500 ppm groups, respectively.

The analytical data indicated that the homogeneity, concentration, and stability of glufosinate-ammonium in the diet preparations were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. Maternal animals: Females were observed twice daily for mortality and moribundity. Clinical signs were recorded once daily for each female from GD 0 until necropsy.

Females presumed to be pregnant (25 per group) were observed outside the home cage for a modified functional observational battery (FOB) at least twice during the gestation period (days 6 and 13) and lactation period (days 10 and 21). The examiner was unaware of the animal's group assignment. No details were provided on the arena size, examination procedures, or scoring criteria. Animals were evaluated for ease of removal from the cage and ease of manual handling and the following functional observations outside the home cage were evaluated:

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe (note: scoring criteria not given) 2) Presence or absence of piloerection and exophthalmos 3) Ranking or count of 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.

Individual maternal body weight and food consumption were measured on GDs 0 and 6-20 and LDs 1-21; group means were calculated. Interval (GDs 6-9, 9-12, 12-15, 15-20, and 6-20) and cumulative (GD 0-20 and LD 1-21) mean body weight gain was calculated for these same animals. Body weight was also recorded when animals were removed from their cages for behavioral assessments. Food consumption was measured on GD 0 and daily on GDs 6-20 and LDs 1-21 and was reported as g/animal/day and g/kg/day.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as PND 0. When parturition was complete, the number of stillborn and live pups in each litter was recorded, and the pups were sexed and examined for gross malformations. Pups were also sexed on PND 4, 11 and 21. All pups were observed once daily for clinical signs of toxicity. A detailed physical examination was conducted on PND 4, 11 and 21 and at weekly intervals thereafter until necropsy.

On PND 4, litters were standardized to a maximum of 10 pups/litter (5/sex/litter, when possible). Culled pups were weighed, euthanized on PND 4 and discarded. If a litter consisted of less than eight pups or did not meet the sex ratio criteria (at least 4/sex), the litter was necropsied on PND 5, and the carcasses were discarded. Due to increased postnatal mortality in the 4500 ppm offspring, two litters that did not meet the sex ratio criteria were maintained.

Surviving pups were weighed on PNDs 1, 4, 7, 11, 13, 17 and 21 and weekly thereafter until necropsy (PND 72) and whenever they were removed from their cages for behavioral testing.

2) **Developmental landmarks:** Beginning on postnatal day 35, male offspring were examined daily for preputial separation. Beginning on postnatal day 25, female offspring were examined daily for vaginal patency. The age of onset and the offspring body weight at that time were recorded.

3) **Postweaning observations:** After weaning on PND 21, offspring were examined by cage-side observations once daily and detailed weekly observations. Individual offspring body weight data were recorded weekly.

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

   i) **Functional observational battery (FOB):** On PNDs 4, 11, 21, 35, 45, and 60, 20 offspring/sex/group were examined outside the home cage in a modified FOB assessment. The same parameters assessed in the maternal FOB were examined in offspring, as appropriate for the developmental stage being observed. Details were not provided on the arena size or examination procedures.

   ii) **Motor activity testing:** Motor activity was evaluated in 20 pups/sex/dose on PNDs 13, 17, 21 and 61. The same animals were tested at each interval. Activity was measured using the SDI Photobeam Activity System, composed of a series of infrared photobeams surrounding a clear, plastic rectangular cage. Data were collected at 5-minute intervals for 60 minutes; but 15-minute interval data were reported . Data for ambulatory and total motor activity were recorded. Total motor activity was defined as a combination of fine motor skills (interruption of a single photobeam) and ambulatory motor activity (interruption of two or more consecutive photobeams).

   iii) **Auditory startle response:** Auditory startle response testing was performed on 20 offspring/sex/dose on PNDs 20 and 60 using the SR-Lab Startle Response System. The same animals were tested at each interval. Each isolation chamber was composed of a wood core measuring 15x16x23 inches and covered with laboratory-grade plastic laminate. Each cabinet was equipped with an internal light, a fan, two viewing lenses and a white-noise generation system. The animal was placed in a cylindrical enclosure of appropriate size, which was then placed into the isolation cabinet. Each enclosure was equipped with a motion sensor.

Testing was performed in a room equipped with a white-noise generation system set to operate at 70 decibels (dB). Each test session consisted of a five-minute acclimation period with a 65-dB broadband background white noise. The startle stimulus for each trial was a 115-dB mixed-frequency noise burst stimulus of approximately 20 millisec in duration. Responses were recorded during the first 100 millisec following the onset of the startle stimulus for each trial. Each session consisted of 50 trials with an eight-second inter-trial interval. Startle response measurements included maximum response amplitude (V_{max}), average response amplitude (V_{ave}) and latency to Vmax (T_{max}), which were analyzed in five blocks of 10 trials each.

iv) **Learning and memory testing:** Learning and memory testing was performed in 20 offspring/sex/dose using a water-filled, eight-unit T-maze similar to that described by Biel[1]. Animals were required to traverse the maze and escape by locating a platform hidden beneath the water surface. The amount of time required and the number of errors were recorded. An error was defined as any instance when an animal deviated from the correct channel with all four feet.

The testing intervals were on PNDs 22 and 62; the same animals were not tested on each day. The testing intervals consisted of three phases conducted over seven consecutive days. For phase one, which was performed on day one of the Biel maze procedure, animals were placed in a straight channel opposite the escape platform, and the time required for each animal to escape was recorded. Each animal was given four trials to assess swimming ability and motivation.

In phase two, which was conducted on days 2-6 of the Biel maze procedure, animals were allowed two trials per day for two consecutive days to solve the maze in path A. Animals were then allowed two trials per day for three consecutive days to solve the path B maze (reverse of path A). For each trial, an animal was allowed three minutes to solve the maze; it was removed at the end of the allotted time if it was not successful. The minimum inter-trial interval was one hour.

In phase three, which was conducted on day 7 of the Biel maze procedure, memory was tested by challenging the animal to solve the maze in path A. Each animal was given two trials to solve the maze.

The maze data were evaluated as the mean time to escape over all trials for each of the three phases (i.e., swimming ability and motivation, sequential learning and memory). The number of errors was evaluated for phases two and three.

2. **Postmortem observations:**

a. **Maternal animals:** Females that did not deliver were euthanized by carbon dioxide inhalation on post-mating day 25. The abdominal and thoracic cavities were opened and

[1] Biel, W.C. (1940). Early age differences in maze performance in the albino rat. J. Genet. Psych. 56:439-453.

examined and the pregnancy status was determined. The numbers of implantation sites and corpora lutea were recorded, if macroscopically evident. Uteri without macroscopic evidence of implantation were opened and placed in a 10% ammonium sulfide solution for detection of early implantation loss, and the carcasses were discarded.

Females with total litter loss were euthanized by carbon dioxide inhalation within 24 hours of litter loss. The abdominal and thoracic cavities were opened and examined. The number of former implantation sites was recorded, and the carcasses were discarded.

Females with litters of fewer than 8 pups or those that failed to meet the sex ratio criteria (at least 4 pups/sex) were euthanized by carbon dioxide inhalation on LD 5 and subjected to gross necropsy. The number of implantation sites was recorded, and the carcasses were discarded. The offspring were euthanized by intraperitioneal injection of sodium pentobarbital and subjected to gross necropsy; the carcasses were discarded. Two females and their litters from the 4500 ppm group were maintained on study, despite the failure to meet the sex ratio criteria, due to increased postnatal mortality in this group.

All females with viable pups on LD 21 were euthanized by carbon dioxide inhalation. The thoracic, abdominal and pelvic cavities were opened, and the contents examined. The number of former implantation sites was recorded, a gross necropsy was performed, and the carcasses were discarded.

b.<u>Offspring</u>: Offspring not selected for behavioral evaluations were euthanized by carbon dioxide inhalation on PND 28 and subjected to gross necropsy. Tissues were retained only if deemed necessary by the gross findings, and the carcass was discarded. On PND 72 after acquisition of sexual development, offspring not allotted for neuropathology/brain weight measurement were euthanized by carbon dioxide inhalation and subjected to necropsy which included examination of the external surfaces, all orifices and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Tissues were retained only if deemed necessary by the gross findings and the carcasses were discarded.

On PND 21, one male or female offspring from each litter (10/sex/group) was euthanized by carbon dioxide inhalation and perfused *in situ* with 4% paraformaldehyde/1.4% glutaraldehyde. The whole brain (including olfactory bulbs) was removed, weighed and the size (length and width) recorded. Abnormal coloration or lesions of the brain and spinal cord were recorded. All brains were prepared for histopathological examination by embedding in paraffin, sectioning and staining with hematoxylin and eosin. Sections from all major brain regions (olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum) were examined. Morphometric measurements were made on Levels 1, 3 and 5 of the brain. Pair-wise matching between measured groups was performed to achieve comparable sections. Level 1 was a coronal section of rostral cerebrum (cerebral cortex, caudoputamen, etc.). Level 3 was a coronal section of mid-cerebrum (cerebral cortex, hippocampal formation, thalamus, etc.). Level 5 was a mid-sagittal section of cerebellum and pons. Measurements of Level 5 were not paired since the other half of these tissues was sectioned transversely for visualization of the cerebellar nuclei. Measurements were

made on homologous sections to ensure that dimensions of the regions were comparable. If there was a deformation or irregularity of a region, morphometric analyses could not be made.

On PND 72, one male and one female from each litter (10/sex/group) was randomly selected from the pups involved in neurobehavioral testing and were euthanized by carbon dioxide inhalation and perfused as on PND 21. The whole brain (including olfactory bulb) was removed, weighed and the size (width and length) recorded. Abnormal coloration or lesions of the brain and spinal cord were recorded. The central nervous system and the peripheral nervous tissues were embedded in paraffin and plastic, respectively. Tissues were sectioned and stained with hematoxylin and eosin. Morphometric measurements were made on Levels 1, 3 and 5 of the brain, as described for PND 21. The following tissues from control and high-dose animals perfused *in situ* at study termination (PND 72) were examined microscopically:

	CENTRAL NERVOUS SYSTEM				PERIPHERAL NERVOUS SYSTEM
	BRAIN				**SCIATIC NERVE**
X	Forebrain (olfactory bulbs)	X	Hippocampus	X	Cross section
X	Cerebral cortex	X	Basal ganglia	X	Longitudinal section
X	Midbrain	X	Thalamus		**OTHER**
X	Cerebellum	X	Hypothalamus	X	Sural Nerve
X	Pons			X	Tibial Nerve
X	Medulla oblongata			X	Peroneal Nerve
	SPINAL CORD			X	Lumbar dorsal root ganglion
X	Cervical swelling			X	Lumbar dorsal root fibers
X	Lumbar swelling			X	Lumbar ventral root fibers
	OTHER			X	Cervical dorsal root ganglion
	Gasserian ganglion			X	Cervical dorsal root fibers
X	Trigeminal ganglion/nerves			X	Cervical ventral root fibers
X	Optic nerve				
X	Eyes			X	Skeletal muscle (gastrocnemius)

D. DATA ANALYSIS:

1. **Statistical analyses:** Analyses were conducted at a significance level of 5% comparing each treated group to the control group by sex with two-tailed group comparisons. The following parameters were subjected to a parametric one-way Analysis of Variance (ANOVA) to determine intergroup differences: mean maternal and offspring body weights, maternal food consumption, length of gestation, implantation sites, unaccounted sites, number of pups born, live litter size, percent of males per litter, day of acquisition of balanopreputial separation or vaginal patency, body weight on day of acquisition, ambulatory count, brain weight, dimensions of F_1 pups, brain morphometric data, FOB, acoustic startle response, and Biel maze data. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the treated groups to the control group. FOB data which yielded scalar and descriptive data were analyzed using the Fisher's Exact Test.

The non-parametric Kruskal-Wallis test with Mann-Whitney U test was used to analyze mean litter proportions (percent per litter). Qualitative histopathological findings were analyzed using the Fisher's Exact Test.

Intra-session total counts for locomotor activity were analyzed by the univariate repeated measures ANOVA (RANOVA) using a Geisser-Greenhouse adjusted F-statistic to determine the presence of an interaction effect of treatment with time. If a significant interaction effect of treatment with time was found using the RANOVA, a parametric one-way ANOVA and Dunnett's test using a Geisser-Greenhouse adjusted F-statistic were used to compare the control and treated groups at each within-session interval. The RANOVA was also used to determine the presence of a main effect of treatment and if indicated, Dunnett's test was used to compare the control and treated groups. Statistical analyses of locomotor activity total counts were performed by BioSTAT Consultants.

2. **Indices:**

   a. **Reproductive indices:** No reproductive indices were calculated.

   b. **Offspring viability indices:** The following viability (survival) indices were calculated:

$$\text{Mean Live Litter Size} = \frac{\text{Total \# of viable pups on PND 0}}{\text{\# of litters with viable pups PND 0}}$$

$$\text{Postnatal Survival Between Birth and PND 4 (\% per litter)} = \frac{\sum [\text{Viable pups per litter on PND 4 (pre-cull)/ Pups born per litter}]}{\text{No. of litters per group}} \times 100$$

$$\text{Postnatal Survival at All Other Intervals (\% Per Litter)} = \frac{\sum (\text{Viable pups per litter at end of interval N/ Viable pups per litter at start of interval N})}{\text{No. of litters per group}} \times 100,$$

where N = PND 0-1 and 4 (post-cull)-21

Indices calculated by the reviewer included the following:

$$\text{Live birth index (\%)} = \frac{\text{number of live born pups at birth}}{\text{number of pups born}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{number of live pups on day 4 pre-cull}}{\text{number of live pups on day of birth}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4 post-cull}} \times 100$$

3. **Positive and historical control data:** Historical control data on male and female Crl:CD®(SD)IGS BR rats from WIL Laboratories included the following: 1) an array of parental reproductive and neonatal endpoints from up to 32 studies by various routes of administration from 1996-2000; 2) acoustic startle response data on PNDs 20/21 (9 studies)

and 60 (10 studies); 3) motor activity data on PNDs 13 (4 studies), 17 (4 studies), 21 (14 studies), 23 (2 studies) and 60/61 (16 studies); 4) morphometric measurements from up to 5 studies on PNDs 11, 21, 22 and 72. However, no procedural information for the studies was included in the report, nor was the date when each study was conducted (behavioral and morphometric studies).

Individual morphometry data on 10 males and 10 females on PND 72 from each of three previous studies was included; each data set included a control and one or three dose groups. No information was given regarding tissue processing or dates of study conduct.

Positive control data were included for inter-observer reliability in the FOB (age of animals not reported); FOB (pups), auditory startle, locomotor activity, learning and memory, and neuropathology and morphometry (pups); optimization of locomotor activity sessions (young adults); acoustic startle response (young adult); and motor activity (young adult). Details and a review of these studies are in the Appendix. WIL Research Laboratories, Inc. demonstrated proficiency only in conducting motor activity and auditory startle tests in young adult rats. Data for the other studies were insufficient for evaluation of proficiency.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No parental females died or were sacrificed moribund during the study.

   Clinical signs in parental females were limited to light-colored feces in the 4500 ppm group. The finding was noted primarily between GDs 8 and 13 and not after LD 2. The number of animals affected was 0/25, 2/25, 4/25 and 8/25 in the control, 200, 1000 and 4500 ppm groups, respectively. This finding, although substance-related, was not regarded as toxicologically significant.

   No treatment-related clinical signs were observed during the FOB on GDs 6 and 13 or LDs 10 and 21. Exophthalmos was observed in 1/25 females in the 4500 ppm group on GD 6 and in 1/25, 1/25, 5/25 and 4/25 animals in the control, 200, 1000 and 4500 ppm groups, respectively, on GD 13. On LD 10, eye prominence was normal in all of these animals and on LD 21, 1/22 females each in the 200 and 1000 ppm groups had exopthalmos. The finding was not considered treatment-related based on the lack of a dose response. Piloerection was observed in 1/25 females at 4500 ppm on GD 13 only. Because of the transient nature and low incidence of the effect, the finding was not considered toxicologically significant.

2. **Body weight and food consumption:** Selected group mean body weight, body weight gain and food consumption values for pregnant or nursing dams are summarized in Table 2. Beginning on GD 7 and continuing throughout gestation, mean body weight in the 4500 ppm group was decreased by 4-8% ($P<0.01$), relative to controls. Negative mean body weight gain was observed in all treated groups at the beginning of treatment (GDs 6-7 and 6-9). Thereafter, significant decreases in weight gain, relative to control, were observed only in the 4500 ppm group during GDs 15-20 (21%) and 6-20 (27%). However, non-statistically

significant decreases were observed in the 200 ppm (8%) and 1000 ppm (10%) groups during the treatment period of gestation (GDs 6-20).

Mean body weight was decreased by 6-11% of control values (P<0.01) in the 4500 ppm group throughout lactation, except on LD 21. Decreases (5-6% of control value) were observed in the 1000 ppm group on LDs 2, 3, 4 and 9 (P<0.05). Cumulative (LD 1-21) mean body weight gain was **increased** by 5%-37% in treated groups compared to the control group during lactation.

Mean food consumption (g/animal/day) during gestation was significantly decreased in all treated groups for GDs 6-9, in the 1000 and 4500 ppm groups for GDs 9-12, in the 200 and 4500 ppm groups for GDs 15-20. Mean food consumption during the treatment period of gestation (GDs 6-20) was decreased by 8% and 17% in the 1000 ppm (P<0.01) and 4500 ppm (P<0.01) groups, respectively. Mean food consumption was decreased throughout lactation (LDs 1-21) by 14% (P<0.01) in the 4500 ppm group and by 5% (nss) in the 1000 ppm group.

TABLE 2. Selected mean (±SD) maternal body weight, body weight gain and food consumption[a]

Observations/study interval	Dietary concentration (ppm)			
	0	200	1000	4500
Gestation (n=24-25)				
Body wt. gestation day 0 (g)	257 ± 16.8	255 ± 13.3	255 ± 14.0	254 ± 13.5
Body wt. gestation day 6 (g)	287 ± 16.0	287 ± 13.1	286 ± 15.6	284 ± 15.4
Body wt. gestation day 13 (g)	318 ± 21.9	313 ± 14.4	313 ± 18.1	300 ± 16.2 (6) **
Body wt. gestation day 20 (g)	400 ± 26.4	391 ± 22.1	388 ± 30.1	367 ± 22.3 (8) **
Wt. gain gestation days 0-6 (g)	30 ± 13.1	31 ± 5.6	31 ± 5.9	30 ± 10.1
Wt. gain gestation days 6-7 (g)	4 ± 7.7	-1 ± 3.6 *	-2 ± 3.4 **	-6 ± 5.8 **
Wt. gain gestation days 6-20 (g)	113 ± 20.1	104 ± 12.5 (8)	102 ± 18.8 (10)	83 ± 13.0 (27) **
Food consumption gestation days 6-20 (g/animal/day)	24 ± 2.0	23 ± 1.6	22 ± 2.1 (8) **	20 ± 1.9 (17) **
Lactation (n=24-25)				
Body wt. lactation day 1 (g)	300 ± 22.9	293 ± 19.4	287 ± 19.2	273 ± 18.4 (9) **
Body wt. lactation day 9 (g)	328 ± 24.6	318 ± 18.7	311 ± 21.8 (5) *	295 ± 18.7 (10) **
Body wt. lactation day 13 (g)	335 ± 24.3	327 ± 22.6	326 ± 23.7	310 ± 18.4 (7) **
Body wt. lactation day 21 (g)	339 ± 20.3	333 ± 20.0	329 ± 24.4	324 ± 18.3 (4)
Wt gain lactation days 1-21 (g)	38 ± 15.1	40 ± 14.4	40 ± 24.2	52 ± 17.0 *
Food consumption lactation days 1-21 (g/animal/day)	59 ± 3.8	57 ± 3.9	56 ± 5.9 (5)	51 ± 8.0 (14) **

[a]Data obtained from pp. 108-131
* Statistically significant different from control, p≤0.05.
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % decrease, relative to control value, calculated by reviewer.

3. **Test substance intake:** Based on maternal food consumption, body weight, and nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight per day during gestation were 14, 69 and 292 for the 200, 1000 and 4500 ppm groups, respectively. During lactation, the mg/kg/day doses were 36, 176 and 756 for the 200, 1000 and 4500 ppm groups, respectively.

4. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. The fertility index was 100% for the control and high-dose groups and 96% for the low-dose and mid-dose groups. The mean duration of gestation was significantly increased in the 4500 ppm group. The mean gestation length was 21.6, 21.8, 21.8 and 22.0 days in the control, 200, 1000 and 4500 ppm groups, respectively. The increase in gestation length at 4500 ppm was not regarded as toxicologically significant, because it fell within the historical control range (21.6-22.3). In addition, 2/25 and 3/25 females had total litter losses at 0 and 4500 ppm, respectively, between PND 1 and 6.

TABLE 3. Reproductive performance[a]

Observation	Dietary concentration (ppm)			
	0	200	1000	4500
Number mated	25	25	25	25
Number pregnant	25	24	24	25
Fertility index (%) [b]	100.0	96.0	96.0	100.0
Intercurrent deaths	0	0	0	0
Mean (±SD) gestation duration (days)	21.6 ± 0.50	21.8 ± 0.44	21.8 ± 0.44	22.0 ± 0.20 **
Number with total litter loss	2	0	0	3

[a] Data obtained from p. 139.
[b] Calculated by the reviewer.
** Statistically significantly different from control, p≤ 0.01.

5. **Macroscopic findings:** No adverse macroscopic findings were observed in any dose group. However, 2-3 (out of 24-25) dams per dose group were not examined at necropsy. No explanation was provided.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) of pups during lactation are summarized in Table 4. No treatment-related effect on the mean number of pups born, mean live litter size and percentage of males per litter was observed. A statistically significant decrease (9%) in postnatal survival occurred on PNDs 0-1 in the 4500 ppm group. The decrease was due to total litter losses for two females and the death of 10 pups in a third litter. Postnatal survival was non-significantly decreased (15%), relative to controls, in the 4500 ppm group for the PND 0-4 interval, primarily due to the losses on PNDs 0-1, but also due to the loss of six pups from a third dam on PNDs 2-4 and total litter loss for a fourth female on PND 3. The viability index (calculated by the reviewer) was 81.6% in the 4500 ppm group compared to 94.4% in the control group. The lactation index (calculated by the reviewer) in the treated groups was comparable to the control. The total number of pups found dead during the pre-weaning period was 31, 13, 17 and 60 in the control, 200, 1000 and 4500 ppm groups, respectively. The number of pups cannibalized in the respective groups was 7, 7, 12 and 35; the majority were missing on PND 1. Pup mortality from PND 0-4 at 4500 ppm was considered treatment-related.

During lactation, an increased number of pups in the 1000 and 4500 ppm groups were observed to be small in size, with 8, 10, 32 and 26 affected in the control, 200, 1000 and

4500 ppm groups, respectively. The incidence of other clinical signs was comparable between the treated and control groups.

TABLE 4. Litter size and viability [a]

Observation	Dietary concentration (ppm)			
	0	200	1000	4500
Total number born	382	360	358	391
Pups/dam delivered	15.3 ± 2.4	15.0 ± 2.7	14.9 ± 3.4	15.6 ± 2.2
Number of litters	25	24	24	25
Number that failed to deliver	0	1	1	0
Number with liveborn litters	25	24	24	25
Number born live	377	353	353	376
Number born dead	5	7	5	15
Number of total litter losses	2	0	0	3
Number surviving to PND 4 (pre-selection)	356	342	335	307
Number pups (post-selection)	236	235	224	213
Number surviving to PND 21	219	228	217	204
Live litter size (PND 0)	15.1 ± 2.4	14.7 ± 2.6	14.7 ± 3.3	15.0 ± 2.4
Sex ratio (% males/litter)	50.3 ± 14.6	52.7 ± 15.6	50.4 ± 11.1	50.5 ± 13.3
Percentage of litter survival				
PND 0-1	96.7 ± 15.3	97.8 ± 5.0	99.0 ± 3.6	87.9 ± 28.5 (9) *
PND 0-4 (pre-selection)	93.7 ± 19.8	94.9 ± 6.5	93.6 ± 17.4	79.7 ± 34.5 (15)
PND 4-21 (post-selection)	91.3 ± 28.2	95.0 ± 20.4	90.4 ± 28.1	95.9 ± 15.3
Live Birth Index (%)[b]	98.7	98.1	98.6	96.2
Viability Index (%.)[b]	94.4	96.9	94.9	81.6
Lactation Index (%)[b]	92.8	97.0	96.9	95.8

[a] Data obtained from pages 140-147 and 795-798.
[b] Calculated by the reviewer
* Statistically significant different from control, $p \leq 0.05$.
Number in parentheses is % decrease, relative to control value, calculated by reviewer.

2. **Body weight:** Selected mean pre-weaning pup body weight and body weight gain data are presented in Table 5. Beginning on PND 4 and continuing throughout the lactation period, mean body weights were significantly decreased, relative to controls, in the 4500 ppm male (13-19%) and female (15-20%) offspring. Mean body weights were also decreased in the 1000 ppm males (8-10% of control values) and females (8-11% of control values) with statistically significant differences achieved on days 7 (females), 13 and 21. Mean body weight gain was significantly decreased from PND 1 in the 4500 ppm male (12-35% of control values) and female (9-36% of control values) offspring, with statistical significance achieved at the PND 1-4, 4-7, 7-11 and 17-21 intervals. Mean weight gain was also

decreased in the 1000 ppm males (6-23% of control values) and females (9-23% of control values) with statistical significance achieved only for the PND 4-7 interval.

TABLE 5. Selected mean (±SD) Pre-weaning pup body weight and body weight gain [a]

PND	Dietary concentration (ppm)							
	0	200	1000	4500	0	200	1000	4500
	Males				Females			
Body weight (g)								
1	6.7±0.6	6.8±0.7	6.8±0.8	6.2 ±0.8 (7)	6.3±0.7	6.5±0.6	6.4±0.8	5.9±0.8 (6)
4 [b]	9.0±1.3	9.3±0.9	8.8±1.7	7.8±1.1 (13)**	8.6±1.2	8.9±0.9	8.5±1.5	7.3±1.1 (15)**
7	14.4±1.7	14.1±1.7	12.9±2.5 (10)	11.7±2.1 (19)**	13.8±1.6	13.9±1.8	12.3±2.6 (11)*	11.0±2.1 (20)**
11	22.4±2.8	21.9±2.7	20.4±3.8 (9)	18.5±2.7 (17)**	21.6±2.6	21.3±3.1	19.4±4.1 (10)	17.3±3.0 (20)**
13	26.7±3.0	25.7±3.2	24.2±4.3 (9)*	22.3±2.9 (16)**	25.8±2.8	24.9±3.7	23.1±4.5 (10)*	20.9±3.1 (19)**
17	35.3±3.6	33.9±3.5	32.5±4.9 (8)	29.8±3.7 (16)**	33.8±3.1	32.8±4.2	31.2±5.1 (8)	28.3±3.5 (16)**
21	45.1±4.8	43.8±4.5	40.9±6.7 (9)*	36.9±4.7 (18)**	43.4±4.6	42.3±4.9	39.3±6.7 (9)*	35.3±3.8 (19)**
Body weight gain (g)								
1-4	2.3±0.9	2.4±0.5	2.0±1.1 (13)	1.5±0.7 (35)**	2.2±0.9	2.4±0.5	2.0±0.9 (9)	1.4±0.7 (36)**
4-7	5.3±1.0	4.9±1.1	4.1±1.7 (23)**	3.9±1.1 (26)**	5.2±0.9	5.0±1.1	4.0±1.7 (23)**	3.7±1.1 (29)**
7-11	8.0±1.6	7.7±1.4	7.5±1.6 (6)	6.6±1.1 (17)**	7.8±1.6	7.4±1.6	7.1±1.8 (9)	6.3±1.1 (19)**
11-13	4.3±0.8	3.8±1.1	3.8±0.8	3.8±0.8 (12)	4.2±0.7	3.6±1.1	3.7±0.9 (12)	3.6±0.5 (14)
13-17	8.6±1.3	8.2±1.0	8.3±1.2	7.5±1.8 (13)	8.0±1.1	8.0±1.1	8.1±1.0	7.3±1.3 (9)
17-21	9.8±2.4	9.9±2.1	8.4±2.4 (14)	7.1±1.6 (28)**	9.5±2.4	9.5±2.2	8.2±2.4 (14)	7.0±1.5 (26)**

[a] Data obtained from pages 216-222.
[b] Before standardization (culling).
N=22-24
* Statistically significantly different from control, p≤ 0.05
** Statistically signficantly different from control, p≤0.01
Number in parentheses is % decrease, relative to control value, calculated by reviewer

Selected mean post-weaning pup body weight and body weight gain data are presented in Table 6. Mean post-weaning (PNDs 28-70) body weight was decreased in males (6-9% of control values) and females (7-10% of control values) at 4500 ppm with a significant difference achieved on days 42, 49 and 56 for males and 49, 56, 63 and 70 for females. Post-weaning body weight gain (days 28-70) was non-significantly decreased in males (6% of control value) and statistically significantly decreased in females (9% of control value) at 4500 ppm.

TABLE 6. Selected mean (±SD) Post-weaning pup body weight and body weight gain [a]				
PND	Dietary concentration (ppm)			
	0	200	1000	4500
Males				
Body Weight (g)				
28	77.6±9.9	78.5±10.1	75.5±12.3	70.9±8.0 (9)
35	136.9±16.9	136.5±14.3	130.9±20.0	124.8±11.3 (9)
49	259.6±25.2	253.8±21.9	244.7±27.7	237.0±17.3 (9)*
70	392.6±32.9	380.9±24.7	374.1±32.2	367.3±25.2 (6)
Body weight Gain (g)				
28-35	59.3±7.5	58.0±6.1	55.4±9.1	53.9±4.8 (9)
35-42	61.8±7.5	60.2±7.4	57.6±8.3	56.9±6.6 (8)
42-49	60.9±6.3	57.1±6.8	56.3±5.4	55.2±6.0 (9)*
28-70	315.0±25.8	302.3±22.0	298.7±28.4	296.4±23.3 (6)
Females				
Body weight (g)				
28	73.7±9.8	74.4±11.2	68.4±13.5	66.0±8.7 (10)
35	117.6±18.8	120.9±12.6	112.6±18.1	109.4±12.9 (7)
49	181.1±17.9	182.1±15.2	169.5±19.7	166.3±14.3 (8)*
70	243.6±20.9	247.2±19.2	230.3±23.1	220.6±16.4 (9)**
Body weight gain (g)				
28-35	44.0±14.9	46.4±4.4	44.3±6.1	43.4±7.3
35-42	36.1±6.5	37.7±5.9	34.1±8.9	32.5±6.3 (10)
42-49	27.4±5.6	23.6±6.4	22.8±6.8	24.4±6.4 (11)
28-70	169.9±17.0	172.8±18.7	161.9±17.6	154.6±14.7 (9)*

[a] Data obtained from pages 223-228.
N = 18-20
* Statistically significantly different from control, $p \leq 0.05$.
** Statistically significantly different from control, $p \leq 0.01$.
Number in parentheses is % decrease, relative to control value, calculated by reviewer.

3. Developmental landmarks:

a. Sexual maturation: There were no adverse effects on the average ages of onset of preputial separation in males or vaginal opening in females (Table 7).

TABLE 7. Mean (±SD) age (days) and body weight (g) at sexual maturation [a]				
Parameter	Dietary concentration (ppm)			
	0	200	1000	4500
N (M/F)	38/39	39/41	40/39	39/38
Preputial separation				
mean age	44.8±2.3	45.9±3.1	45.9±3.1	46.2±2.6
mean body weight	225.8±18.3	232.7±19.4	224.3±19.4	217.4±17.8
Vaginal opening				
mean age	33.5±2.2	33.8±3.1	34.8±4.0	34.5±3.2
mean body weight	107.7 ± 12.0	112.0 ± 20.3	112.3 ± 21.2	107.7 ± 18.1

[a] Data obtained from pages 232 and 236.

4. **Behavioral assessment:**

a. **Functional observational battery:** No treatment-related FOB changes were observed at any of the testing periods (PNDs 4, 11, 21, 35, 45 and 60).

b. **Motor/locomotor activity:** Total motor and ambulatory activity data are presented in Table 8. Interval data are presented in Tables 9 (males) and 10 (females). The total session and interval counts within groups varied greatly with large standard deviations and coefficients of variation ranging from 50% to greater than 100%. In addition, because the range of historical control values was so large (a 1000-2000 count difference was often recorded for total motor activity), motor activity for treatment groups was compared to concurrent controls only.

On PND 13, mean total and ambulatory values were non-significantly increased (1.8 and 2.5x control value, respectively) in males at 4500 ppm. On PND 17, total and ambulatory counts were significantly increased (2.5x control value) in males at 4500 ppm and non-significantly increased (2.2 and 2.6x control value, respectively) in females at 4500 ppm; the counts in males exceeded the upper value of the historical control range. There were also non-significant increases in total and ambulatory counts in females at 200 ppm (1.5-1.8x control value) and 1000 ppm (1.7-1.9x control value).

On PND 21, total and ambulatory counts were significantly increased in males (2.2-2.5x control value) and females (2.0-2.9x control value) at 1000 ppm and in males (2.6-2.8x control value) and females (1.8-2.2x control value) at 4500 ppm. Total counts in males at 4500 ppm exceeded the upper value of the historical control range. Total and ambulatory counts in females at 1000 and 4500 ppm exceeded the upper values of the historical control range. Total and ambulatory counts were non-significantly increased in males (1.5-1.9x control value) and females (1.2-1.4x control value) at 200 ppm.

On PND 61, total and ambulatory counts in the 1000 and 4500 ppm groups remained slightly increased (1.2-1.3x control value) in males and statistically significantly increased (1.3-1.5x control value) in females. Mean ambulatory counts were statistically significantly increased at 200 ppm.

Statistically significant increases in individual intervals were observed on PND 21 in males (0-15 minutes: all doses; 16-30 minutes: 1000 and 4500 ppm; 31-45 minutes: 4500 ppm); on PND 21 in females (0-15 and 31-45 minutes: 1000 and 4500 ppm; 16-30 minutes: 1000 ppm); and on PND 61 in males (0-15 minutes: 4500 ppm). Although increased mean motor activity was observed at several intervals at 200 ppm, the variability around the means was too high to consider the observations adverse.

Test day	TABLE 8: Mean total and ambulatory motor activity counts (±SD)[a]				
	Dietary concentration (ppm)				Mean Historical Control Range
	0	200	1000	4500	
Males					
PND 13					
Total	363 ± 266.2	505 ± 400.6	434 ± 360.9	651 ± 647.5 (79)	336-1354
Ambulatory	94 ± 132.7	155 ± 231.5	140 ± 186.6	237 ± 331.3 (151)	49-514
PND 17					
Total	800 ± 887.0	1128 ± 938.9	682 ± 499.4	2003 ± 1029.0 (150)**	425-1872
Ambulatory	305 ± 426.7	426 ± 388.5	201 ± 232.7	767 ± 509.4 (151)**	146-731
PND 21					
Total	398 ± 275.2	597 ± 384.6 (50)	874 ± 566.6 (120)**	1044 ± 514.8 (162)**	267-923
Ambulatory	109 ± 99.8	206 ± 227.5 (89)	277 ± 233.6 (154)*	304 ± 174.4 (179)**	78-416
PND 61					
Total	1606 ± 583.0	1858 ± 461.1 (16)	1953 ± 689.0 (22)	1916 ± 655.3 (19)	1232-2322
Ambulatory	516 ± 256.0	609 ± 187.7	658 ± 290.3	668.0± 258.5 (29)	373-818
Females					
PND 13					
Total	427 ± 382.6	448 ± 479.8	356 ± 201.1	418 ± 327.8	247-1224
Ambulatory	123 ± 234.1	146 ± 229.2	70 ± 74.7	93 ± 152.6	46-492
PND 17					
Total	550 ± 576.6	846 ± 634.1 (54)	927 ± 777.9 (69)	1190 ± 841.5 (116)	190-2256
Ambulatory	173 ± 243.0	303 ± 281.8 (75)	326 ± 342.2 (88)	451 ± 405.9 (161)	38-934
PND 21					
Total	496 ± 194.0	601± 414.8 (21)	985 ± 686.8 (99)*	893 ± 483.3 (80)*	279-699
Ambulatory	128 ± 68.2	181 ± 122.9 (41)	372 ± 411.5 (91)**	280 ± 168.4 (119)	84-209
PND 61					
Total	1414 ± 408.1	1772 ± 598.1	1948 ± 490.7 (38)*	1879 ± 379.8 (33)*	1136-2152
Ambulatory	496 ± 177.9	663 ± 264.3 (34)*	747 ± 234.1 (51)**	736 ± 172.2 (48)**	409-863

[a] Data were obtained from pages 257-264 of the study report.
N=18-20.
Number in parentheses is % increase, relative to control value, calculated by reviewer.

Interval (min)	TABLE 9: Mean (± SD) sub-session motor activity in males (# movements/15-minute interval)[a]				
	Dietary concentration (ppm)				Mean Historical Control Range
	0	200	1000	4500	
PND 13					
0-15	94 ± 70.2	147 ± 151.5	123 ± 96.8	181 ± 148.5 (93)	122-276
16-30	103 ± 121.5	181 ± 155.4	100 ± 112.7	170 ± 239.6 (65)	100-362
31-45	92 ± 99.4	89 ± 123.4	144 ± 148.1	161 ± 195.3 (75)	52-327
46-60	74.0 ± 75.8	89 ± 122.0	67 ± 104.2	139 ± 134.5 (88)	62-389
PND 17					
0-15	281 ± 232.1	284 ± 222.5	247 ± 147.4	539 ± 242.6 (92)	187-675
16-30	245 ± 284.0	316 ± 262.4	237 ± 228.4	530 ± 364.5(116)	110-452
31-45	173 ± 296.0	263 ± 276.9	147 ± 220.7	499 ± 330.1 (188)	47-381
46-60	101 ± 215.5	266 ± 342.2	51 ± 71.7	435 ± 346.4 (331)	13-364
PND 21					
0-15	257 ± 145.7	415 ± 134.4 (61)*	508 ± 187.7 (98)*	487 ± 122.3 (89)*	192-472
16-30	46 ± 75.7	71 ± 121.2 (54)	181 ± 181.2 (293)*	241 ± 188.3 (424)*	22-217
31-45	39 ± 63.7	41 ± 109.7	107 ± 150.0 (174)	168 ± 154.9 (331)*	9-201
46-60	56 ± 126.8	71 ± 136.2	78 ± 156.6	148 ± 157.3 (164)	8-147
PND 61					
0-15	817 ± 213.8	831 ± 168.1	934 ± 233.0	983 ± 190.7 (20)*	692-1105
16-30	332 ± 169.5	392 ± 185.3	475 ± 199.8 (43)	468 ± 222.8 (41)	256-501
31-45	226 ± 150.4	321 ± 173.5	311 ± 220.1 (38)	264 ± 193.5 (17)	51-350
46-60	231 ± 185.7	314 ± 206.2	233 ± 206.3	201 ± 186.3	32-371

[a] Data were obtained from pages 257-260 of the study report.
N = 18-20.
* Treatment group by trial block interaction effect (p=0.0014 and 0.049 for PND 21 and 61, resp.); significantly different from the control group at α=0.05 using Dunnett's test.
Number in parentheses is % increase, relative to control value, calculated by reviewer.

Interval (min)	TABLE 10: Mean (± SD) sub-session motor activity in females (# movements/15-minute interval) [a]				
	Dietary concentration (ppm)				Mean Historical Control Range
	0	200	1000	4500	
PND 13					
0-15	99 ± 80.2	96 ± 71.7	123 ± 88.0	113 ± 74.2	54-313
16-30	132 ± 131.8	133 ± 188.8	92 ± 88.6	108 ± 102.9	41-367
31-45	105 ± 140.7	152 ± 226.3	65 ± 64.3	113 ± 199.2	64-292
46-60	92 ± 147.8	67 ± 117.4	76 ± 56.1	83 ± 89.4	82-252
PND 17					
0-15	224 ± 146.8	330 ± 181.2 (47)	296 ± 159.9 (32)	437 ± 262.3 (95)	106-716
16-30	104 ± 166.2	220 ± 199.4 (112)	245 ± 271.9 (136)	344 ± 275.6 (231)	20-564
31-45	109 ± 183.7	179 ± 197.9 (64)	222 ± 275.2 (104)	265 ± 244.2 (143)	34-534
46-60	112 ± 181.9	118 ± 186.7	164 ± 258.4 (46)	144 ± 188.4 (29)	30-442
PND 21					
0-15	319 ± 130.0	423 ± 145.0 (33)	469 ± 182.4 (47)*	483 ± 153.6 (51)*	185-416
16-30	65 ± 81.8	76 ± 119.2	223 ± 240.5 (243)*	129 ± 137.8 (98)	12-126
31-45	31 ± 41.9	48 ± 86.4	197 ± 248.0 (535)*	161 ± 154.9 (419)*	19-161
46-60	81 ± 120.9	54 ± 136.8	96 ± 143.7	120 ± 161.5	11-99
PND 61					
0-15	815 ± 140.2	887 ± 191.9	985 ± 150.2	960 ± 134.2	797-1047
16-30	255 ± 196.5	410 ± 223.4	488 ± 244.8	461 ± 161.6	221-498
31-45	180 ± 141.2	282 ± 250.4	317 ± 183.0	289 ± 202.5	45-340
46-60	164 ± 160.6	193 ± 174.5	158 ± 159.4	169 ± 163.7	36-301

[a] Data were obtained from pages 261-264 in the study report.
N = 19-20.
* Treatment group by trial block interaction effect (p=0.011 for PND 21); significantly different from the control group at α=0.05 using Dunnett's test.
Number in parentheses is % increase, relative to control value, calculated by reviewer.

c. **Auditory startle reflex habituation:** Overall maximum response amplitude (V_{max}), latency to V_{max} (T_{max}), and average response amplitude (V_{ave}) data in F_1 male and female rats are presented in Table 11. Mean interval response amplitude and latency to peak in F_1 male and female rats are presented in Table 12. No statistically significant differences between treated and control groups were observed. Habituation (decrease in response amplitude over the course of the test session) could not be determined due to the variability around the mean values for each trial period.

TABLE 11: Mean (±SD) overall (Blocks 1-5) acoustic startle peak amplitude (mv), latency to peak (msec) and average response amplitude (mv) [a]

Dietary conc. (ppm)	Parameter	Males		Females	
		PND 20	PND 60	PND 20	PND 60
0	V_{max} (mv)	94.9±38.2	106.1±55.3	100.7±52.0	57.8±23.3
	T_{max} (msec)	27.8±4.2	32.5±6.1	25.1±2.5	34.0±3.6
	V_{ave} (mv)	21.5±8.8	23.6±11.9	22.0±10.4	12.5±4.9
200	V_{max} (mv)	102.7±54.6	113.2±89.7	122.7±50.0	98.0±54.3
	T_{max} (msec)	25.7±3.7	35.4±7.7	24.7±3.3	33.0±4.9
	V_{ave} (mv)	22.6±11.8	25.6±20.0	25.7±8.6	21.1±10.7
1000	V_{max} (mv)	92.5±28.6	133.4±107.0	98.1±46.6	86.0±50.9
	T_{max} (msec)	27.0±3.6	31.3±5.1	26.9±3.9	33.0±5.7
	V_{ave}(mv)	20.7±6.3	29.5±24.0	21.8±10.7	18.3±11.1
4500	V_{max} (mv)	105.7±34.7	131.1±92.6	108.5±64.6	89.8±97.4
	T_{max} (msec)	24.9±3.1	32.5±5.4	25.2±4.8	32.8±5.7
	V_{ave} (mv)	22.3±7.5	27.7±19.7	23.0±12.1	18.8±19.8

[a] Data obtained from pages 237-248 in the study report.
N=18-20
V_{max} =maximum response amplitude
T_{max} =latency to V_{max}
V_{ave} = average response amplitude

TABLE 12: Mean (±SD) interval acoustic startle peak amplitude (mv) and latency to peak (msec) in F_1 male and female rats [a]

Dietary conc. (ppm)	Parameter	Trials 1-10	Trials 11-20	Trials 21-30	Trials 31-40	Trials 41-50
Males - PND 20						
0	V_{max} (mv)	131.7±42.1	92.3±42.3	89.5±38.4	85.0±54.3	75.0±46.6
	T_{max} (msec)	28.7±6.1	27.9±6.8	27.0±4.9	27.5±6.0	27.8±6.1
200	V_{max} (mv)	120.9±65.9	98.6±54.4	104.4±61.5	98.4±57.3	91.0±56.9
	T_{max} (msec)	28.6±7.5	24.5±3.9	24.9±4.3	26.0±4.8	24.7±3.7
1000	V_{max} (mv)	114.6±45.7	93.4±37.6	93.5±44.0	76.5±26.6	84.6±37.1
	T_{max} (msec)	29.6±6.9	27.1±5.6	24.1±2.8	26.3±4.8	28.0±5.8
4500	V_{max} (mv)	124.8±62.9	101.4±35.1	99.0±34.0	97.3±35.0	106.1±36.7
	T_{max} (msec)	27.3±3.2	24.9±4.7	24.6±4.9	24.3±4.7	23.6±4.6
Males - PND 60						
0	V_{max} (mv)	147.0±89.4	118.7±73.0	93.8±64.5	79.5±58.7	91.2±75.2
	T_{max} (msec)	29.8±6.0	30.7±6.5	33.5±9.3	34.2±9.5	34.2±9.9
200	V_{max} (mv)	177.0±146.9	111.3±110.8	90.1±78.5	102.5±90.1	85.2±74.2
	T_{max} (msec)	31.5±7.6	35.9±8.3	34.6±7.2	36.5±12.6	38.6±12.6
1000	V_{max} (mv)	187.9±132.9	148.0±145.1	118.2±133.1	105.9±96.8	107.0±73.1
	T_{max} (msec)	31.2±5.8	30.8±6.9	33.1±8.0	30.6±5.6	30.9±7.5
4500	V_{max} (mv)	191.6±140.8	169.1±147.7	102.9±83.5	110.9±111.1	81.1±62.7
	T_{max} (msec)	30.3±6.8	31.6±7.2	34.1±7.8	32.1±8.5	34.4±5.8
Females - PND 20						
0	V_{max} (mv)	123.7±64.9	103.0±62.7	96.9±58.0	87.7±54.0	92.0±44.0
	T_{max} (msec)	27.2±4.7	24.2±3.5	25.6±4.4	24.6±4.0	23.8±4.8
200	V_{max} (mv)	148.2±73.1	121.1±58.1	110.0±54.8	119.9±53.4	114.2±50.4
	T_{max} (msec)	27.1±6.1	25.6±5.0	24.5±4.4	23.2±4.5	22.9±4.1
1000	V_{max} (mv)	128.8±71.5	94.0±49.3	83.6±41.7	100.2±53.3	83.9±45.9
	T_{max} (msec)	29.2±5.7	27.1±5.6	26.8±4.4	25.2±5.5	26.4±4.6
4500	V_{max} (mv)	125.6±63.2	103.2±56.8	98.0±64.7	110.0±80.3	105.7±73.0
	T_{max} (msec)	27.1±7.5	26.3±6.0	25.8±6.1	23.5±5.1	23.1±4.9
Females - PND 60						
0	V_{max} (mv)	83.0±36.9	57.5±32.7	48.0±22.6	53.6±28.7	46.7±25.4
	T_{max} (msec)	33.3±8.0	32.9±6.5	34.3±6.4	34.1±5.5	35.3±6.9
200	V_{max} (mv)	132.5±70.4	101.6±80.4	87.7±58.4	89.6±73.6	78.5±66.7
	T_{max} (msec)	30.0±5.2	33.4±8.1	33.2±7.1	34.3±6.1	33.9±9.3
1000	V_{max} (mv)	113.0±51.3	87.7±70.1	85.2±68.3	77.4±65.2	66.7±35.5
	T_{max} (msec)	31.3±6.5	32.0±9.2	33.2±7.2	36.1±7.9	32.3±8.2
4500	V_{max} (mv)	149.1±153.4	86.5±95.5	83.6±103.4	65.2±75.1	64.8±78.1
	T_{max} (msec)	31.2±5.2	31.7±5.3	31.2±9.6	34.6±8.4	35.5±8.9

[a] Data obtained from pages 237-247 in the study report.

N=18-20

V_{max} =maximum response amplitude

T_{max} =latency to V_{max}

d. Learning and memory testing:

Watermaze performance: The watermaze performance data for PNDs 22 and 62 are presented in Tables 13 and 14, respectively. The mean time to escape from the Biel maze and mean number of errors for Trials 1-10 (days 2-6) were similar for all groups, indicating no treatment-related effect on learning. The mean time to escape and mean number of errors were similar across dose when pups were probed for memory ability (day 7). No significant differences were noted, except on PND 22 when the day 1 swimming time was significantly increased in males at 1000 ppm. The difference is not considered treatment-related since a dose response was not observed.

TABLE 13. Water maze performance (mean ± SD) in offspring on PND 22 [a]

Day/Trial	Dietary concentration (ppm)			
	0	200	1000	4500
Males				
Day 1 - Swimming Ability Mean Time (secs)	9.27±2.98	8.75±2.64	13.79±7.40**	10.92±2.76
Trial 1 (Day 2) - *Path A* Mean Time (secs) Mean No. Errors	75.43±46.78 12±9.0	71.21±44.24 12±8.3	105.26±56.50 19±9.7	96.25±45.50 18±8.6
Trial 2 (Day2) - Path A Mean Time (secs) Mean No. Errors	95.80±38.62 19±8.8	58.20±41.59 11±9.7	85.23±62.37 14±10.8	58.34±42.23 10±8.9
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	50.61±29.97 11±7.9	79.04±60.56 20±18.4	59.25±39.48 13±12.4	54.58±48.49 12±11.6
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	46.51±27.54 10±7.1	46.93±42.49 10±13.9	45.89±39.31 9±8.1	66.20±53.25 14±13.3
Trial 5 (Day 4) - *Path B* Mean Time (secs) Mean No. Errors	158.51±38.38 32±10.6	140.93±48.28 25±11.1	138.73±54.50 22±10.3	155.63±47.40 25±10.6
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	115.32±61.03 23±12.8	113.93±66.07 21±13.9	106.23±60.52 18±12.3	130.32±56.87 24±13.1
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	104.93±56.54 23±14.7	107.33±66.67 20±12.5	94.24±65.57 17±12.6	107.98±64.69 20±13.3
Trial 8 (Day 5) - Path B Mean Time (secs) Mean No. Errors	84.29±59.20 16±11.4	78.61±55.84 13±10.0	97.44±63.67 16±12.5	95.55±70.91 16±13.5
Trial 9 (Day 6) - Path B Mean Time (secs) Mean No. Errors	69.24±52.68 13±11.7	80.30±64.54 14±13.7	75.38±62.12 14±12.7	76.54±66.37 11±10.9
Trial 10 (Day 6) - Path B Mean Time (secs) Mean No. Errors	71.35±58.74 12±11.5	63.71±58.56 10±10.4	59.83±55.24 10±12.2	75.85±74.91 11±14.0
Trial 11 (Day 7) - *Path A (Probe)* Mean Time (secs) Mean No. Errors	80.72±42.79 21±12.6	78.03±42.31 16±9.7	72.75±45.30 17±11.9	76.97±58.15 18±16.9
Trial 12 (Day 7) - Path A (Probe) Mean Time (secs) Mean No. Errors	55.63±38.21 12±9.5	46.08±24.34 10±7.8	48.58±35.91 10±7.9	56.13±46.82 12±11.6

GLUFOSINATE AMMONIUM/128850 Day/Trial	Dietary concentration (ppm) OPPT 870.6300/ OECD 426			
	0	200	1000	4500
Females				
Day 1 - Swimming Ability Mean Time (secs)	11.10±5.25	10.64±3.73	10.97±3.35	11.50±5.71
Trial 1 (Day 2) - *Path A* Mean Time (secs) Mean No. Errors	76.53±49.74 14±10.7	83.95±48.77 15±9.1	69.14±38.95 12±7.3	84.35±46.81 16±11.1
Trial 2 (Day 2) - Path A Mean Time (secs) Mean No. Errors	77.99±46.23 12±7.5	79.06±61.66 13±12.2	63.37±49.20 12±10.5	65.86±26.69 12±5.7
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	64.53±48.28 13±11.0	66.07±37.14 14±8.5	69.96±52.55 16±12.8	62.93±34.61 14±9.6
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	50.25±41.29 9±7.8	62.82±48.81 13±9.91	39.05±20.32 7±5.0	51.28±30.46 11±9.4
Trial 5 (Day 4) - *Path B* Mean Time (secs) Mean No. Errors	123.22±61.68 25±15.0	126.57±56.94 22±10.1	150.09±48.68 26±9.5	120.95±63.06 19±10.0
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	128.94±65.39 25±14.2	108.25±61.33 19±11.6	94.96±56.60 17±11.3	109.44±62.66 20±11.5
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	102.33±58.75 21±13.2	104.88±65.83 19±13.2	74.79±55.22 13±9.8	94.03±70.57 16±14.2
Trial 8 (Day 5) - Path B Mean Time (secs) Mean No. Errors	80.63±56.87 14±11.5	105.40±61.09 18±11.9	65.21±51.96 10±9.5	92.70±68.60 16±13.8
Trial 9 (Day 6) - Path B Mean Time (secs) Mean No. Errors	86.77±73.03 16±14.5	81.10±62.16 14±12.7	42.14±36.21 7±6.0	56.80±39.68 10±8.5
Trial 10 (Day 6) - Path B Mean Time (secs) Mean No. Errors	47.64±38.62 7±6.9	82.83±64.64 12±10.2	47.49±47.58 8±9.6	77.35±70.99 12±14.7
Trial 11 (Day 7) - *Path A (Probe)* Mean Time (secs) Mean No. Errors	72.03±41.19 17±12.1	96.34±44.04 23±10.8	60.63±40.83 16±13.5	69.91±32.44 17±11.2
Trial 12 (Day 7) - Path A (Probe) Mean Time (secs) Mean No. Errors	69.06±42.62 15±11.1	65.99±35.50 14±10.8	60.76±38.97 15±11.4	47.22±31.89 8±6.9

[a] Data obtained from pages 273-282 in the study report.

A = Forward route through maze

B = Reverse route through maze

N=19-20

** Statistically significantly different from control, $p<0.01$.

TABLE 14. Water maze performance (mean ± SD) in offspring on PND 62 [a]				
Day/Trial	Dietary concentration (ppm)			
	0	200	1000	4500
Males				
Day 1 - Swimming Ability Mean Time (secs)	6.15±1.77	7.36±3.84	6.59±2.81	6.11±1.61
Trial 1 (Day 2) - *Path A* Mean Time (secs) Mean No. Errors	77.65±57.01 15±11.6	88.22±55.88 18±11.3	65.43±39.20 15±9.7	68.93±47.89 13±8.8
Trial 2 (Day2) - Path A Mean Time (secs) Mean No. Errors	44.69±23.28 9±6.3	57.63±43.92 12±8.7	44.91±44.31 10±12.5	40.26±35.0 8±6.8
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	26.86±17.91 5±4.7	47.26±50.08 9±10.3	55.28±46.85 14±13.3	44.92±38.17 11±9.5
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	28.34±28.48 5±8.6	26.04±18.17 4±4.0	28.55±19.44 6±6.0	19.19±8.93 3±2.6
Trial 5 (Day 4) - *Path B* Mean Time (secs) Mean No. Errors	108.37±65.52 21±14.1	129.73±55.22 19±10.2	155.43±39.56 31±13.5	128.85±59.91 22±13.5
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	89.14±61.63 15±11.7	92.11±63.08 14±10.6	108.09±57.64 21±10.9	94.93±65.76 17±12.1
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	74.91±63.23 11±9.7	64.76±48.90 10±9.0	76.56±54.74 11±7.6	61.25±48.38 9±7.7
Trial 8 (Day 5) - Path B Mean Time (secs) Mean No. Errors	45.42±51.20 6±8.0	45.14±48.97 7±7.8	36.27±39.78 5±5.6	23.79±18.53 3±3.4
Trial 9 (Day 6) - Path B Mean Time (secs) Mean No. Errors	42.12±4.69 7±10.4	48.51±58.26 7±10.4	42.15±31.91 7±7.7	20.52±23.77 3±5.5
Trial 10 (Day 6) - Path B Mean Time (secs) Mean No. Errors	24.87±28.74 4±7.6	35.38±40.69 4±5.5	22.68±12.40 3±3.2	18.32±16.65 2±4.4
Trial 11 (Day 7) - *Path A (Probe)* Mean Time (secs) Mean No. Errors	54.33±35.75 10±6.4	61.09±46.77 12±11.1	55.03±34.95 12±5.4	60.39±32.13 13±7.6
Trial 12 (Day 7) - Path A (Probe) Mean Time (secs) Mean No. Errors	45.28±38.91 9±9.5	26.99±12.75 6±5.5	38.78±34.09 9±7.1	22.98±17.52 3±4.3

Day/Trial	Dietary concentration (ppm)			
	0	200	1000	4500
Females				
Day 1 - Swimming Ability Mean Time (secs)	7.31±2.97	6.82±1.91	6.26±2.54	6.20±1.10
Trial 1 (Day 2) - *Path A* Mean Time (secs) Mean No. Errors	59.59±37.57 15±9.2	76.56±36.26 15±7.9	62.02±46.06 14±9.7	80.75±60.40 15±11.6
Trial 2 (Day 2) - Path A Mean Time (secs) Mean No. Errors	38.03±21.62 8±7.2	53.47±31.04 12±8.7	46.52±22.51 12±7.5	53.41±36.79 11±8.7
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	31.01±17.14 6±5.8	34.01±22.91 7±6.3	34.50±21.72 8±7.2	42.62±39.92 10±12.5
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	19.65±6.28 2±2.2	27.20±17.48 4±4.9	25.29±13.90 4±3.5	23.31±18.80 4±6.0
Trial 5 (Day 4) - *Path B* Mean Time (secs) Mean No. Errors	121.16±59.26 25±14.1	119.24±62.19 20±11.8	136.53±49.87 27±14.4	105.74±59.85 17±11.2
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	72.22±57.00 14±13.5	83.99±66.78 14±12.7	88.74±65.06 16±12.7	88.86±57.61 17±10.8
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	80.68±60.65 14±12.3	68.23±50.72 10±8.6	64.50±58.89 10±10.9	66.71±45.89 11±9.3
Trial 8 (Day 5) - Path B Mean Time (secs) Mean No. Errors	39.53±41.08 6±8.0	41.73±29.94 6±4.5	27.09±18.63 4±4.1	24.22±17.83 3±3.9
Trial 9 (Day 6) - Path B Mean Time (secs) Mean No. Errors	43.18±47.44 8±10.2	23.54±14.44 3±3.0	26.76±39.56 5±9.7	31.36±26.01 6±7.9
Trial 10 (Day 6) - Path B Mean Time (secs) Mean No. Errors	23.42±18.38 3±6.5	20.05±14.56 2±3.1	22.29±23.96 2±3.6	24.30±32.23 3±6.6
Trial 11 (Day 7) - *Path A (Probe)* Mean Time (secs) Mean No. Errors	41.84±25.46 8±7.0	51.08±32.84 10±8.1	45.32±20.80 9±6.2	39.73±18.99 7±4.6
Trial 12 (Day 7) - Path A (Probe) Mean Time (secs) Mean No. Errors	39.73±31.13 8±7.1	34.93±25.64 5±4.9	24.49±11.91 3±3.5	35.48±30.91 7±9.2

*Data obtained from pages 283-292 in study report.

A = Forward route through maze

B = Reverse route through maze

N=19-20

7. Postmortem results:

a. **Brain weight:** Mean absolute brain weight data are presented in Table 15. No significant differences were observed in absolute brain weights between treated and control groups.

TABLE 15. Mean (±SD) Absolute and Relative Brain Weight Data in Offspring [a]

Parameter	Dietary concentration (ppm)			
	0	200	1000	4500
Males - Day 21				
Brain weight (g)	1.662±0.066	1.627±0.063	1.636±0.095	1.641±0.091
Males - Day 72				
Brain weight (g)	1.959±0.154	1.900±0.070	1.848±0.112	1.917±0.049
Females - Day 21				
Brain weight (g)	1.613±0.073	1.563±0.151	1.590±0.107	1.570±0.156
Females - Day 72				
Brain weight (g)	1.830±0.106	1.817±0.129	1.792±0.173	1.750±0.101

[a] Data obtained from pages 299-300 and 303-304 in the study report.
N=10
* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
Percent change, relative to controls, in parentheses.

b. **Macroscopic examination:** Necropsy of pups found dead during the pre-weaning period revealed no treatment-related gross findings. No remarkable changes were found in culled pups euthanized on PND 5 (because of litters failing to meet the sex ratio). At the PND 28 scheduled necropsy, no treatment-related macroscopic findings were observed. In the 1000 ppm group, a dilated right renal pelvis was observed in 1 male/119 pups (1/24 litters), while an absent left testis and small left epididymis were observed in a second male (1/24 litters). One female (out of 133 pups; 1/24 litters) in the 200 ppm group had a herniated diaphragm, while 1 male/133 pups (1/24 litters) in this group had a yellow polycystic mass on the right kidney and a distended right ureter with white precipitate. One control group female had a dilated right renal pelvis. No gross findings were noted in the brain or spinal cord.

In the animals euthanized following acquisition of sexual development landmarks, no treatment-related findings were observed. An accessory liver lobe was observed in 1/20 females in the 1000 ppm group. In the 200 ppm group males, single incidences of dark red discoloration of the thymus and of small testes and epididymides were observed. In the control group, 1/20 females had pale lungs and a second had bilateral dilated renal pelvis.

At the PND 72 necropsy of offspring not selected for neuropathology and brain weighing, the only macroscopic finding was dilated renal pelvis in 1/10 males at 200 ppm. However, macroscopic examination was only performed for 8/10 control males and 9/10 females at 1000 and 4500 ppm. In animals selected for brain weighing (at PND 21 and 72), no gross findings were noted in the brain or spinal cord. However, macroscopic examination (PND 72) was only performed for 9/10 control females and 9/10 males at 4500 ppm.

c. **Neuropathology:**

1) **Microscopic examination:** Select microscopic findings are found in Table 16. No treatment-related microscopic observations were observed on PNDs 21 or 72. The incidences of any findings showed no relation to dosing and were therefore regarded as not toxicologically significant. Findings at PND 21 included hypercellularity (minimal) in the cerebral cortex immediately bordering the rostral dorsal lateral ventricles, malaligned (minimal) hippocampal pyramidal cells, and persistent external granule cell layer in the cerebellum.

On PND 72, malaligned pyramidal neurons in the hippocampus and abnormal foliation of the cerebellum occurred with comparable frequency in control and treated groups. Minimal or mild (one control male) axonal degeneration of the sciatic nerve characterized by digestion chambers containing myelin debris was common in both sexes of treated and control groups. One female at 4500 ppm had neuronal cell bodies (ectopic tissue) in the sural nerve. Microscopic examination was only performed for 9/10 control females and 9/10 males at 4500 ppm.

TABLE 16. Incidence of Select Microscopic Findings in Offspring [a]

Parameter	Dietary concentration (ppm)			
	0	200	1000	4500
Males - Day 21				
Cerebral cortex Minimal hypercellularity	0/10	1/10	1/10	0/10
Cerebellum Minimal persistent external granule cell layer	2/10	2/10	3/10	2/10
Males - Day 72				
Hippocampal pyramidal cells Malaligned (minimal)	6/10	3/10	1/10	3/9
Sciatic nerve Axonal degeneration Minimal Mild	 7/10 1/10	 0/10 0/10	 0/10 0/10	 7/9 0/9
Tibial nerve Axonal degeneration Minimal	1/10	0/10	0/10	2/9
Peroneal nerve Axonal degeneration Minimal	2/10	0/10	0/10	3/9
Cerebellum Minimal abnormal foliation	2/10	0/10	0/10	1/9
Females - Day 21				
Cerebral cortex Minimal hypercellularity	1/10	1/10	0/10	1/10
Cerebellum Minimal persistent external granule cell layer	2/10	2/10	2/10	0/10
Hippocampal pyramidal cells Malaligned (minimal)	2/10	0/10	0/10	1/10
Females - Day 72				

Hippocampal pyramidal cells				
Malaligned (minimal)	4/9	0/10	1/10	3/10
Sciatic nerve				
Axonal degeneration				
Minimal	4/9	0/10	0/10	5/10
Sural nerve				
Axonal degeneration				
Minimal	0/9	0/10	0/10	2/10
Ectopic tissue				
Minimal	0/9	0/10	0/10	1/10
Tibial nerve				
Axonal degeneration				
Minimal	1/9	0/10	0/10	2/10
Cerebellum				
Minimal abnormal foliation	3/9	0/10	0/10	2/10
Cerebral cortex				
Mild dilatation	0/9	0/10	0/10	1/10
Hypothalamus				
Mild dilatation	0/9	0/10	0/10	1/10

[a] Data obtained from pages 307-311 and 314-25 in the study report.

2) **Brain Morphometry:** Data on morphometric measurements are presented in Table 17. Initial examination of the control and high-dose males for PND 21, revealed a slight decrease (approximately 5%) in mean vertical height between the layer of pyramidal neurons in the Level 3 hippocampal formation at 4500 ppm. Therefore, the brain sections from the 200 and 1000 ppm group were prepared and examined in both sexes. At this examination, the mean vertical thickness of the cortex on Level 1 for males at 200 and 1000 ppm was significantly increased, and the mean width of the base of the cerebellar lobule 9 on Level 5 for females at 200 ppm was significantly increased compared to the control. These findings were not considered toxicologically significant due to the lack of a dose-response relationship.

In the PND 72 examinations, significantly decreased (11% of control value) mean vertical height between the layers of pyramidal neurons in the hippocampal formation in Level 3 was observed in males at 4500 ppm. A non-significant decrease (7% of control value) in this measurement was observed in males at 1000 ppm. The values in both the 1000 and 4500 ppm groups were outside the historical control range. Females at 4500 ppm had significantly decreased (9% of control value) radial thickness of the cortex in Level 3, which was slightly outside the historical control range. A dose-dependent decrease in mean length of the ventral limb of the dentate hilus (Level 3) was observed in both males (9-15%) and females (12-20%) at ≥200 ppm. Statistical significance was reached in females at 200 ppm.

TABLE 17. Mean (±SD) brain morphometric measurements (cm) [a]

Parameter	Dietary concentration (ppm)				Mean Historical Control Range (cm)
	0	200	1000	4500	
Males - Day 21					
Brain length (mm)	17.7±0.3	18.1±0.5	17.8±0.6	17.3±0.5	---

Parameter	Dietary concentration (ppm)				Mean Historical Control Range (cm)
	0	200	1000	4500	
Brain width (mm)	14.1±0.3	14.1±0.3	14.3±0.6	14.1±0.5	---
Level 1 V Thickness cortex	0.160±0.011	0.193±0.022**	0.191±0.016**	0.163±0.009	0.155-0.249
Level 3 Radial thickness cortex	0.136±0.009	0.143±0.006	0.142±0.015	0.134±0.008	0.127-0.163
V Ht BTW Hippocampal Pyramidal Neuron Layers	0.081±0.004	0.086±0.007	0.079±0.008	0.077±0.003	0.075-0.107
Length ventral limb dentate hilus	0.124±0.016	0.123±0.009	0.117±0.022	0.113±0.010	0.117-0.122
Level 5 Base of lobule 9	0.062±0.007	0.068±0.009	0.067±0.007	0.064±0.005	0.058-0.060
Males - Day 72					
Brain length (mm)	22.7±2.7	23.6±2.9	22.7±3.0	22.1±2.9	—
Brain width (mm)	14.9±0.8	14.9±0.6	15.0±0.5	14.9±0.4	---
Level 1 V Thickness cortex	0.178±0.018	0.166±0.012	0.164±0.008	0.166±0.011	0.154-0.184
Level 3 Radial thickness cortex	0.145±0.014	0.141±0.008	0.140±0.013 (4)	0.136±0.015 (6)	0.128-0.155
V Ht BTW Hippocampal Pyramidal Neuron Layers	0.099±0.008	0.096±0.008	0.092±0.006 (7)	0.088±0.007 (11)*	0.093-0.096
Length ventral limb dentate hilus	0.154±0.022	0.140±0.014 (9)	0.136±0.016 (12)	0.131±0.017 (15)*	0.136-0.164
Level 5 Base of lobule 9	0.075±0.012	0.070±0.005	0.068±0.008	0.068±0.010	0.067-0.070
Females - Day 21					
Brain length (mm)	17.3±0.5	17.2±1.0	17.1±0.6	16.8±0.6	---
Brain width (mm)	14.2±0.5	14.3±0.6	14.1±0.4	13.7±0.5	---
Level 1 V Thickness cortex	0.175±0.016	0.197±0.029	0.164±0.010	0.172±0.021	0.153-0.235
Level 3 Radial thickness cortex	0.132±0.006	0.137±0.010	0.134±0.007	0.130±0.012	0.140-0.150
V Ht BTW Hippocampal Pyramidal Neuron Layers	0.078±0.003	0.082±0.006	0.081±0.005	0.079±0.008	0.079-0.096
Length ventral limb dentate hilus	0.108±0.009	0.120±0.005	0.118±0.018	0.114±0.015	0.117-0.121
Level 5 Base of lobule 9	0.058±0.006	0.067±0.009*	0.058±0.003	0.061±0.006	0.058-0.063
Females - Day 72 [b]					
Brain length (mm)	22.7±2.8	22.5±2.9	22.2±2.8	22.0±2.7	---
Brain width (mm)	14.8±0.4	14.6±0.3	14.6±0.4	14.4±0.5	---
Level 1 V Thickness cortex	0.159±0.013	0.164±0.012	0.160±0.010	0.157±0.017	0.156-0.188
Level 3 Radial thickness cortex	0.138±0.010	0.134±0.007	0.138±0.008	0.126±0.009 (9)*	0.128-0.144
V Ht BTW Hippocampal Pyramidal Neuron Layers	0.093±0.009	0.088±0.005	0.089±0.009	0.090±0.008	0.092-0.096
Length ventral limb dentate hilus	0.149±0.018	0.131±0.009 (12)*	0.125±0.015 (16)**	0.119±0.011 (20)**	0.127-0.152

Level 5 Base of lobule 9	0.065±0.006	0.067±0.007	0.064±0.006	0.066±0.004	0.065-0.067

[a] Data obtained from pages 299-300, 303-304, 312-313, 326-327 in the study report.
[b] Data from one female in the 4500 ppm group that had dilatation of the lateral ventricles of the cerebral cortex and the third ventricle of the hypothalamus on PND 72 was not included in the calculation of the mean for that group.
Ht = height
V= vertical
BTW = between
N = 9-10
* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
Number in parentheses is % decrease, relative to control, calculated by reviewer

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author established the NOAEL for maternal systemic toxicity at 200 ppm based on decreased body weight gain and food consumption at 1000 and 4500 ppm. The NOAEL for maternal reproductive toxicity (process of parturition and duration of gestation) and neonatal toxicity was 4500 ppm. The NOAEL for developmental toxicity was 200 ppm based on increases in offspring locomotor activity at 1000 ppm.

B. **REVIEWER COMMENTS:** No parental females died during the study. The only clinical sign was light-colored feces, which occurred primarily between GDs 8 and 13 in dams at 4500 ppm and probably indicated elimination of the test article. No treatment-related clinical signs were observed in dams during the FOB. Beginning on GD 7, mean body weight in the 4500 ppm group was significantly decreased due to weight loss after the initiation of treatment and continuing lower body weight gain throughout gestation for this group. Non-significant decreases in weight gain were observed in the 200 ppm and 1000 ppm groups during the treatment period of gestation (GDs 6-20). Mean food consumption during gestation was significantly decreased in all treated groups at the beginning of treatment, most likely due to decreased palatability. During the remainder of gestation, food consumption was significantly decreased in the 1000 and 4500 ppm groups. Mean body weight was slightly decreased in the 1000 and 4500 ppm group during the beginning of lactation; however, overall (LDs 1-21) mean body weight gain was increased in treated groups as compared to the control group. Mean food consumption was significantly decreased throughout lactation in the 4500 ppm groups. The decreases in body weight, body weight gain and food consumption during gestation at 1000 and 4500 ppm are considered treatment-related and toxicologically significant. The effects at 200 ppm were sporadic and minor and therefore are not considered toxicologically significant. No treatment-related findings were observed on gross necropsy.

No effect on the mean number of pups born, mean live litter size or sex ratio per litter was observed. A significant treatment-related decrease in postnatal survival occurred on PNDs 0-1 and PND 0-4 in the 4500 ppm group, due mostly to total litter losses. Increased numbers of pups in the 1000 and 4500 ppm groups were observed to be small in size and correlated with a decrease in body weight and body weight gain for these groups. Lack of an apparent dose response in the number of pups small in size could have been due to the deaths in the high-dose group. The incidence of other clinical signs was comparable between the treated and

control groups. Beginning on PND 4 and continuing throughout the lactation period, mean body weight and body weight gain were decreased in the 1000 and 4500 ppm male and female offspring. Mean post-weaning (PNDs 28-70) body weight and body weight gain were decreased in males and females at 4500 ppm. The average onset of preputial separation in males and vaginal opening in females was not affected by treatment. No treatment-related changes in FOB parameters were observed during any of the testing periods.

Total and ambulatory motor activity was increased in males at 4500 ppm on PNDs 13, 17, and 21, in females at 1000 and 4500 ppm on PNDs 17, 21, and 61, and in males at 1000 ppm on PNDs 21 and 61. Corresponding sub-session interval activity was also increased for these groups on each testing day. Slightly increased activity in the 200 ppm males and females on some testing days was not considered biologically or toxicologically significant.

No statistically significant differences between treated and control groups were observed in auditory startle response. There was no treatment-related effect on learning and memory, since mean time to escape from the maze and mean number of errors were similar for all groups. No significant differences were noted, except on PND 22 when the day 1 swimming time was significantly increased in males at 1000 ppm. The difference was not considered treatment-related since there was no dose response.

Brain weight measurements and gross and microscopic necropsy findings were not affected by treatment. The PND 21 morphometric measurements showed significant increases in the mean vertical thickness of the cortex on Level 1 for males at 200 and 1000 ppm and in the mean width of the base of the cerebellar lobule 9 on Level 5 for females at 200 ppm. The changes are not considered treatment-related, since there was no relation to dosing. For the PND 72 examinations, decreased mean vertical height between the layers of pyramidal neurons in the hippocampal formation in Level 3 was observed in males at 1000 and 4500 ppm; the values in both groups were outside the historical control range. Females at 4500 ppm had significantly decreased (9% of control value) radial thickness of the cortex in Level 3, which was slightly outside the historical control range. A dose-dependent decrease in mean length of the ventral limb of the dentate hilus (Level 3) was observed in both males (9-15%) and females (12-20%) at ≥200 ppm. Statistical significance was reached in females at 200 ppm.

The maternal LOAEL for Glufosinate-ammonium in rats is 4500 ppm (292 mg/kg/day during gestation) based on decreased body weight, body weight gain, and food consumption during gestation and lactation. The maternal NOAEL is 1000 ppm (69 mg/kg/day during gestation).

The offspring LOAEL for Glufosinate-ammonium in rats is 200 ppm (14 mg/kg/day during gestation) based on brain morphometric changes. The offspring NOAEL was not observed.

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 pending review of the of positive control data and because glutamine synthetase measurements in the liver, kidneys,

and brain of dams and pups were not made (Memo, P.V. Shah, July 10, 2002, HED doc. # 0050900).

C. STUDY DEFICIENCIES: The following study deficiencies were noted:

1. A signed and dated Quality Assurance statement was not provided.
2. Coefficients of variation of 50% to over 100% were observed in motor and locomotor activity data.
3. Auditory startle reflex habituation could not be determined due to the variability around the mean values for each trial period.
4. The historical control data were not explained adequately. Appendix J is labeled "WIL Brain Morphometric Historical Control Data (Crl:CD®(SD)IGS BR)" and contains measurements for various sections of Level 1, 2, 3 and 5 of the brain. Data are included on males and females for PNDs 11, 21, 22, and 72 from 1-3 studies. Appendix K is labeled "PND 72 Brain Morphometry Data from Three Previous Studies (WIL Research Laboratories, Inc.)" and includes individual animal data from control and treated animals in Studies A, B and C. It is unclear how these data relate to the summary tables in Appendix J. It is also unclear why data from treated animals were included. In addition, no information was provided on when the studies were conducted, the strain of rat (Appendix K) or dosing method.
5. No explanation was provided as to why 2-3 (out of 24-25) dams per dose group were not examined at necropsy.
6. At the PND 72 necropsy of offspring not selected for neuropathology and brain weighing, macroscopic examination was only performed for 8/10 control males and 9/10 females at 1000 and 4500 ppm. In animals selected for brain weighing, macroscopic examination was only performed for 9/10 control females and 9/10 males at 4500 ppm.
7. At the PND 72 necropsy, microscopic examination was only performed for 9/10 control females and 9/10 males at 4500 ppm.
8. No details were provided on the arena size, examination procedures, or scoring criteria used for maternal or offspring detailed clinical observations (modified FOB).