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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

TXR# 0053629

DATE: February 23, 2006

MEMORANDUM

SUBJECT: **FENAMIDONE** - Review of Developmental Neurotoxicity Study in Rats (MRID 46590001)

PC Code: 046679
DP Barcode #: D319665

From: Robert J. Mitkus, PhD
Registration Action Branch I (RAB1)
Health Effects Division (HED) (7509C)

Thru: P.V. Shah, PhD, Branch Senior Scientist
RAB1
HED (7509C)

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

ACTION REQUESTED: The Registration Division (RD) has requested HED to perform a review of a Developmental Neurotoxicity Study in Rats (MRID 46590001) for fenamidone. This study was required as part of the conditional registration of fenamidone. The action was successfully completed, and the conclusions of the study are reported here.

I. CONCLUSIONS

RAB1 has reviewed the Developmental Neurotoxicity Study in Rats (MRID 46590001) for fenamidone. The study is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426. Previously, the Health Effects Division suggested that a preliminary dose-range finding study be conducted if the Crl:CD(SD)BR strain of rat were not utilized in the required DNT study, since the 2-generation reproduction study (MRID 45400014) was conducted with the Crl:CD(SD)BR strain of rat (Memo, PV Shah, 12-Jan-2004, TXR# 0052296). However, the

DNT study was conducted with the Wistar Hannover strain of rat. It is therefore not possible to assess whether the decreases in absolute brain weight that were observed in the F1 and F2 generations in the 2-generation reproduction study using the Crl:CD(SD)BR strain of rat would also be observed in the required developmental neurotoxicity study, if the Crl:CD(SD)BR strain of rat were used. The submitted study does not satisfy the registration requirement of a Developmental Neurotoxicity Study in Rats.

II. STUDY REVIEWED

Developmental Neurotoxicity Study - Rat; OPPTS 870.6300

CITATION: Sheets, L.P., R.G. Gilmore, and Hoss H.E. (2005) A developmental neurotoxicity screening study with technical grade fenamidone in Wistar rats. Bayer CropScience LP Toxicology, Stilwell, KS. Study Number 04-D72-UM, June 29, 2005. MRID 46590001. Unpublished.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46590001), Fenamidone (99.2% a.i.; batch # OP2250040) was administered in the diet to 30 female mated Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR rats/dose at nominal concentrations of 0, 60, 250, 1000 or 4700 ppm from gestation day (GD) 6-20. Dietary concentrations were reduced to 0/0/0, 34/28/24, 145/116/95, 584/471/388, or 2716/2222/1844 ppm, during weeks 1/2/3 of lactation, respectively, based on estimated increases in feed consumption during lactation. Average doses to the animals throughout the study were 0, 5.5, 23.2, 92.3, or 429 mg/kg/day, respectively. A Functional Operational Battery (FOB) was performed on 30 dams/dose on GDs 13 and 20, and on 10 dams/dose on LDs 11 and 21. On postnatal day (PND) 4, litters were culled to yield four males and four 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 (passive avoidance and watermaze testing), and neuropathology at study termination (day 75±5 of age). 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 by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No maternal deaths or clinical signs of toxicity were observed during the study. FOB testing was unaffected by treatment. Mean body weight during gestation was unaffected by treatment. However, at 4700 ppm mean body weight gain was significantly decreased (8% relative to controls) during GDs 6-20 due to weight gain that was decreased by 27%, relative to controls, during GDs 6-13. Food consumption measurement during GDs 6-13 in the 4700 ppm group was compromised by spillage. Food consumption was decreased at 1000 ppm during GDs 6-13 (7% decrease relative to controls), but without a toxicologically significant change in body weight or body weight gain. Mean body weight during lactation was slightly decreased (3-5% relative to controls) in females at 4700 ppm. Food consumption during lactation was unaffected by treatment. No treatment-related effects were observed on reproductive performance.

Litter viability and clinical signs in offspring were unaffected by treatment. No treatment-related effects were observed at birth; however, body weight was significantly decreased in males (9-10% relative to controls) and females (8-11% relative to controls) at 4700 ppm from PNDs 4-21. Body weight gain during the pre-weaning period was significantly decreased in males (9-20% relative to controls) and females (8-20% relative to controls) at 4700 ppm. Post-weaning (PNDs

28-70) body weight was significantly decreased in males by 5-6%, relative to controls, and females (4-5% relative to controls) at 4700 ppm during the first three weeks. Body weight gain was similar between the treated and control groups during post-weaning. Attainment of vaginal opening in females, preputial separation in males, and pupil constriction response in both sexes was not affected by treatment. No treatment-related effects were observed on FOB, motor/locomotor activity, auditory startle habituation or learning and memory testing (passive avoidance and watermaze performance). No treatment-related effects were reported for ophthalmologic testing. At necropsy, brain weight, macroscopic/ microscopic examination of the nervous system and morphometric measurements were unaffected by treatment.

The maternal systemic and neurotoxicity LOAEL for Fenamidone was not determined. The maternal NOAEL is 4700 ppm (429 mg/kg/day).

The offspring systemic and neurotoxicity LOAEL for Fenamidone in rats is 4700 ppm (429 mg/kg/day) based on decreased body weight (9-11%) and body weight gain (8-20%) during pre-weaning and decreased body weight (4-6%) during post-weaning. The offspring NOAEL is 1000 ppm (92.3 mg/kg/day).

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EPA Reviewer: Robert J. Mitkus, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)
EPA Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: Robert J. Mitkus
Date 2/23/2006
Signature:
Date

TXR#: 0053629

DATA EVALUATION RECORD

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

PC CODE: 046679

DP BARCODE: D319665

TEST MATERIAL (PURITY): Fenamidone (99.2% a.i.)

CHEMICAL NAME: (5*S*)-3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-4*H*-imidazol-4-one

CITATION: Sheets, L.P., R.G. Gilmore, and Hoss H.E. (2005) A developmental neurotoxicity screening study with technical grade fenamidone in Wistar rats. Bayer CropScience LP Toxicology, Stilwell, KS. Study Number 04-D72-UM, June 29, 2005. MRID 46590001. Unpublished.

SPONSOR: Bayer CropScience, Research Triangle Park, NC

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controls) during GDs 6-20 due to weight gain that was decreased by 27%, relative to controls, during GDs 6-13. Food consumption measurement during GDs 6-13 in the 4700 ppm group was compromised by spillage. Food consumption was decreased at 1000 ppm during GDs 6-13 (7% decrease relative to controls), but without a toxicologically significant change in body weight or body weight gain. Mean body weight during lactation was slightly decreased (3-5% relative to controls) in females at 4700 ppm. Food consumption during lactation was unaffected by treatment. No treatment-related effects were observed on reproductive performance.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Fenamidone (technical grade)

Description:	beige powder
Batch #:	OP2250040
Purity:	99.2% a.i.
CAS # of TGAI:	161326-34-7
Structure:	Not available

2. Vehicle: None

3. Test animals:

Species:	Rat
Strain:	Wistar (Crl:WI[Glx/BRL/Han]IGS BR)
Age at cohousing:	Males: 15 weeks; Females: 12 weeks
Source:	Charles River Laboratories, Inc., 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:	Purina Mills Rodent Lab Chow 5002 in meal form *ad libitum*, except during neurobehavioral testing.
Water:	Municipal tap water was available *ad libitum*.
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** 10 per hour **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	6 days

B. STUDY DESIGN:

1. In life dates: Start: January 20, 2004; End: April 30, 2004

2. Study schedule: Mated female Wistar rats (30/dose group) were administered the test material in the diet from gestation day (GD) 6 through lactation day (LD) 21. On postnatal day (PND) 4, litters were standardized to 8 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. Pups remained on study to PND 75.

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

4. Animal assignment: After the acclimation period, dams with body weight more or less than 20% of the mean weight were rejected and sacrificed without necropsy. The remaining females were assigned to the control or treated groups in sequence as they were determined to be inseminated, as shown in Table 1. Parental generation males were only breeders and were arbitrarily selected for co-housing with females.

TABLE 1. Study design					
Experimental parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
Maternal animals					
No. of maternal animals assigned					
No. of maternal animals assigned	30	30	30	30	30
FOB (GDs 13 and 20)	30	30	30	30	30
FOB (LDs 11 and 21)	10	10	10	10	10
Offspring					
Minimum No. of offspring assigned					
Set A - motor activity (PNDs 13, 17, 21 and 60±2)[a]	10/sex	10/sex	10/sex	10/sex	10/sex
Set B - Acoustic startle habituation (PNDs 22 and 60±2)[a]	10/sex	10/sex	10/sex	10/sex	10/sex
Set C - Passive Avoidance (PNDs 22, 29), Water Maze (PND 60±2, 67±2), FOB (PNDs 4, 11, 21, 35±1, 45±1, 60±2)[a]	10/sex	10/sex	10/sex	10/sex	10/sex
Set D - Tissues (PND 21)	10/sex	10/sex	10/sex	10/sex	10/sex
Set A-C - Ophthalmology (PND 50-60), tissues (PND 75±5), brain weight (PND 75±5)	10/sex	10/sex	10/sex	10/sex	10/sex

[a] One male and/or female per litter (approximately 16/sex, representing 20 litters per dose) with a minimum of 10/sex were assigned.

5. **Dose selection rationale:** The dose selection was based on the results of the two-generation reproduction study in Sprague-Dawley (Crl:CD(SD)BR) rats and EPA's revised HIARC document dated April 24, 2003. In the reproduction study, Fenamidone was administered in the diet at nominal concentrations of 0, 60, 1000 and 5000 ppm (achieved doses of 5.2, 84, and 459.6 mg/kg/day in females). The achieved high doses during the pre-mating phase for the F_0 and F_1 adult females were 459.6 and 438.3 mg/kg/day, respectively. Body weight was decreased in F_0 adults (significantly), F_1 offspring, F_1 adults and F_2 pups. Absolute, but not relative, brain weight was significantly decreased at 1000 and 5000 ppm in F_1 adults and F_2 female pups. The NOAEL was 1000 ppm (84 mg/kg/day). As determined in a chronic study in Sprague-Dawley rats, the limit of palatability for Fenamidone in the diet is approximately 5000 ppm. Based on the results of the 2-generation reproduction study and the dietary palatability limit of Fenamidone in the diet, the high dose of 460 mg/kg/day was considered by the registrant to be too high for the developmental neurotoxicity study. Therefore, the highest dietary level was reduced to provide a dose of 400 mg/kg/day to help avoid decreased maternal body weight which would confound the interpretation of findings in offspring (e.g., decreased brain weight and startle response that are secondary to decreased body weight). Thus, the doses selected for this study were 0, 5, 20, 80 and 400 mg/kg/day. In order to maintain more consistent doses to the animals, dietary concentrations of Fenamidone were adjusted during lactation weeks 1, 2, and 3 by factors of 1.9, 2.3, and 2.8, respectively, to avoid a large increase in dose that would occur with increased food consumption.

6. **Dose administration:** Fenamidone was administered in the diet to maternal animals on GD 6 through LD 21. After PND 21, untreated food was provided for all groups. It should be noted that exposure of the pups to fenamidone, e.g., through the milk, was not demonstrated.

7. **Dietary preparation and analysis:** Dietary concentrations were not adjusted to correct for the purity (99.2% a.i.) of the test material. The test material was passed through a #35-mesh sieve and mixed with the basal diet without a solvent.

   The test diets were prepared weekly and stored in the freezer until presentation to the animals. Concentration of the test substance in the diet was measured by liquid chromatography on all five batches of feed used during the study. The stability and homogeneity of the test material in the feed were analyzed using 10 and 5000 ppm concentrations in another study.[1]

Results:

Homogeneity analysis: No data were provided.

Stability analysis: No data were provided.

Concentration analysis: For gestation, the relative-to-nominal 60, 250, 1000 and 4700 ppm concentrations averaged 105%, 108%, 105% and 106%, respectively. During the remaining three weeks of treatment, the average percentage of the nominal concentration was 109%, 108%, 109% and 110% for the respective dose levels.

The analytical data indicated that the concentration of Fenamidone in the diet preparations was adequate. Data on homogeneity and stability should have been included.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Parental generation females were observed cage-side for mortality, moribundity and clinical signs of toxicity at least once daily during the study. A detailed physical examination on females was performed once daily during the dosing period.

   Those females presumed to be pregnant (approximately 30 per group) were observed outside the home cage for a functional observational battery (FOB) at least twice during the gestation period (days 13 and 20). At least 10 dams per group were examined twice during the lactation period (days 11 and 21). The arena size and examination details were not provided. The following functional observations were recorded:

[1] Jensen, T.L., "A Homogeneity and Stability Study of Fenamidone Technical in Rodent Ration", Bayer CropScience LP, Report Number 201324, 2005. No MRID.

Functional observations–Maternal animals	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe; 2) Presence or absence of piloerection and exophthalamus; 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; 6) Respiration; 7) Activity/arousal level.
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 recorded on GDs 6, 13 and 20 and on LDs 0, 7, 14 and 21. Dams were also weighed on LD 4.

b. <u>Offspring</u>:

1) **<u>Litter observations</u>:** The day of completion of parturition was designated as PND 0. The number of pups delivered and the pup status at birth were recorded for each litter. If a dam delivered fewer than three pups per sex or if the litter size decreased to less than seven pups by PND 4, the dam and litter were sacrificed without necropsy. All pups were observed cage-side once daily for mortality, moribundity, clinical signs of toxicity and behavioral changes. Detailed observations for clinical signs were made once daily before weaning and once weekly thereafter.

   On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible) using a random selection technique. If there were more than 23 acceptable litters for any group, the surplus litters were sacrificed on PND 4 after weighing and without necropsy. Culled dams and pups and dams with insufficient litters were sacrificed by CO_2 asphyxiation and decapitation, respectively.

   Surviving pups were weighed on PND 0, 4, 11, 17 and 21 and once weekly thereafter. They were also weighed when vaginal patency and preputial separation were first evident. Food consumption was not measured after weaning on PND 21.

2) **<u>Developmental landmarks</u>:** Beginning on postnatal day 38, 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 at that time was recorded. All pups were also tested for pupil constriction on PND 21.

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

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

i) **Functional observational battery (FOB) (Set C):** On PNDs 4, 11, 21, 35±1, 45±1, and 60±2, a minimum of ten offspring/sex/group representing at least 20 litters/group assigned to "Set C" were examined outside the home cage in a FOB assessment by an individual who was unaware of the group assignment. The same parameters assessed in the maternal FOB were examined in offspring, as appropriate for the developmental stage being observed. Neonates (PNDs 4 and 11) were not evaluated in the open field since the observer did not consider it necessary for the evaluation.

ii) **Motor activity testing (Set A):** Motor activity was evaluated in a minimum of ten pups/sex/dose representing at least 20 litters/group on PNDs 13, 17, 21 and 60±2. Activity was monitored for 60 minutes (six ten-minute intervals) in figure-eight mazes. Each maze consisted of eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys). A Columbus Instruments Universal Maze Monitoring System was used for data collection. Broad-spectrum background noise (74±2 dB) was provided throughout the test to minimize acoustical variation during testing. The uniformity of light intensity over each maze was verified each day. Motor activity was measured as the number of beam interruptions that occurred during each session (one activity count was equivalent to one beam interruption). Locomotor activity was measured by eliminating consecutive counts for a given beam, i.e., until the animal relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrement in activity over consecutive intervals of each session.

iii) **Auditory startle reflex habituation (Set B):** Auditory startle reflex habituation testing was performed on at least ten offspring/sex/dose representing at least 20 litters per group on PNDs 22 and 60±2. Groups of four animals were tested simultaneously within the startle system enclosure (Coulbourn Instruments). The enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material and housed a speaker mounted in the ceiling to provide the eliciting stimulus (a 50-msec burst of white noise at approximately 118 dB). The enclosure housed four load cell/force transducer assemblies that measured the startle response. During the test session, the animals were placed in cages that were positioned on the top of each load cell. Sound measurements were made using a Bruel and Kjaer Real-Time Frequency Analyzer fitted with a microphone. The animals were allowed a 5-minute acclimation period in the enclosure at ambient noise levels before being presented with the startle-eliciting stimulus at 10-sec intervals. Data collection began with the presentation of the stimulus and continued for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (one sample/msec for 200 msec) and converted to grams using a calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's response curve. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of the stimulus. This baseline was taken to represent an approximate body weight measurement to verify that the equipment was functioning properly. Response amplitude was defined as the maximum value of the average curve minus the baseline. Latency to peak was the time (msec)

following the onset of the stimulus when the peak response occurred. A total of 50 trials (5 blocks of 10 trials each) was carried out.

iv) **Learning and memory testing (Set C):** Learning, short-term retention and long-term retention were assessed in a **passive avoidance test** on PNDs 22 and 29. A minimum of ten pups/sex/group representing at least 20 litters/group were tested. Only animals that demonstrated acquisition were tested for retention. Testing was conducted using an integrated system of equipment and computer programs from Coulbourn Instruments. Testing occurred in individual isolation cubicles, each with a single shuttle cage. Each shuttle cage (approximately 14 x 7 inches) was separated into two compartments of equal size by a wall that supported a centrally-located sliding door. The walls of one compartment were lined with black film (dark side) and the walls of the other compartment were illuminated with a high-intensity lamp. After adaptation, the animal was placed into the lighted compartment facing toward the light. After approximately 20 seconds, the light was illuminated and the door between the compartments was opened. When the rat moved into the dark compartment, the door closed, a shock was delivered and the light was switched off. The rat was then returned to its cage until the next trial. If the rat did not cross to the dark compartment within 180 seconds, it was returned to its cage and given a latency score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials elapsed, whichever occurred first. Animals that failed to reach criterion performance with 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase. The test was repeated one week later. In the second trial, rats were placed in the illuminated compartment, given a 20-second acclimation period and then the latency to enter the dark side was recorded. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Learning and memory in a minimum of 10 animals/sex/group representing at least 20 litters per group were also assessed using **water maze testing** on PND 60±2 and again seven days later. The M-maze was constructed of Plexiglas with five-inch-wide corridors and contained approximately 7.5 inches of water maintained at 22±1°C. For each trial, the rat was placed in the starting position at the base of the M-maze stem, located between the two lateral arms. For the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the maze. The initial arm for the learning trial was designated as the incorrect goal for the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any trial were guided to the correct goal with the exit ramp and removed from the maze. Between trials, the animals were kept in a transport cage for approximately 15±5 seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in a test session was fifteen. Latency (time in seconds to choose the correct goal or the maximum of 60 seconds) and the number of errors (incorrect turns in the maze) were recorded for each trial. Only animals that demonstrated acquisition within the 15-trial limit were tested for retention seven days later. Dose groups were compared using the following dependent variables:

Acquisition (First Test): number of trials to criterion (measure of overall learning); average number of incorrect turns in maze for each trial (measure of overall learning); and latency to reach the correct goal on trial 2 (measure of short-term retention).

Retention (Second Test): number of trials to criterion (measure of long-term retention); average number of errors for each trial (measure of long-term retention); and latency to reach the correct goal on trial 1 (measure of long-term retention).

5) **Ophthalmology**: At approximately 50-60 days of age, ophthalmic examinations were conducted on a minimum of 10 rats/sex/group representing at least 20 litters selected for perfusion at study termination. Pupillary reflex was tested using a penlight or transilluminator after dilation of the pupils with a mydriatic. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilation. After dilation, the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

2. **Postmortem observations**:

a. **Maternal animals:** Parental females found moribund were sacrificed by CO_2 asphyxiation. Females found dead or moribund were subjected to gross necropsy and possible collection of tissues at the discretion of the study director.

Following co-habitation, males were sacrificed and discarded. Dams were sacrificed by CO_2 asphyxiation on LD 21 after the weaning of their litters; necropsy was not conducted. Mated females that did not deliver a litter were sacrificed on GD 24 without necropsy.

b. **Offspring**: All moribund pups were sacrificed and subjected to gross necropsy. Tissues were collected at the discretion of the study director. Animals found dead underwent necropsy and were disposed of without collection of tissues. Pups selected for culling were sacrificed by decapitation and discarded without necropsy.

Animals selected for perfusion on PND 21 (Set D) and at study termination (Sets A-C) were anesthesized with an intraperitoneal dose of pentobarbital and then perfused via the left ventricle with a sodium nitrite flush (in phosphate buffer) followed by *in situ* fixation using universal fixative [1% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde] in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves), selected bilateral peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. All tissues were placed in 10% buffered formalin. The brain was weighed upon removal from the skull before placement in the formalin.

Prior to sectioning for histology, the following brain measurements were made using a Vernier caliper: 1) anterior-to-posterior length of the cerebrum, extending from the anterior pole to posterior pole, exclusive of the olfactory bulbs; and 2) anterior-to-posterior length of the cerebellum, extending from the anterior edge of the cortex to the

posterior pole. These measurements were performed by a technician who was aware of the dose assignments.

After gross measurements, the brain was divided into eight coronal sections for microscopic examination. The eight sections were processed for paraffin embedding, sectioned and examined after staining with hematoxylin and eosin (H&E). Brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained with luxol fast blue/cresyl violet. The following tissues were also collected from the perfused animals at terminal necropsy for embedding in paraffin and staining with H&E: three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA), sectioned and stained with a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

Only tissues from control and high-dose animals were examined for micropathology and morphometry. If no lesions were found, the other dose groups were not examined. Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency and severity of each lesion was determined before the code was broken and the data were evaluated for a dose-effect relationship.

Seven linear measurements of selected brain regions were taken, including the two gross measurements on the intact brain discussed above. The other five taken from the histologic sections using software calibrated with an ocular micrometer were as follows:

1. Frontal cortex thickness (forebrain) - measurement of the dorsal portion of the cerebral cortex within a coronal section passing through the region of the optic chiasm.

2. Parietal cortex thickness (forebrain) - measurement of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. Caudate putamen horizontal width (forebrain; maximum cross-sectional width) - measurement on the coronal section at the level of the optic chiasm.

4. Hippocampal gyrus thickness (midbrain) - mean of two measurements of the full width on both sides of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter.

5. Cerebellum height (cerebellum/pons) - measurement extending from the roof of the fourth ventricle to the dorsal surface.

All brain sections from the control and high-dose male and female offspring also underwent micropathologic evaluation.

D. DATA ANALYSIS:

1. **Statistical analyses:** Continuous data were assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. If there were unequal variances, the data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons).

   FOB continuous data were analyzed using an ANOVA, with *post-hoc* comparisons using Dunnett's test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling Procedures, with *post-hoc* comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

   Motor and locomotor activity (total session activity and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data for the four test occasions were analyzed using an ANOVA to determine if there was a significant day-by-treatment interaction. If so, Dunnett's test was used to determine if the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine if there was a significant treatment-by-interval interaction on each test occasion. If so, the data were analyzed using Dunnett's test to determine whether the treated group was significantly different from the control.

   Acoustic startle response amplitude data (peak amplitude) for the treatment groups were first analyzed using an ANOVA. If there was a significant group effect, Dunnett's test was used to determine if a treated group was significantly different from the control. The response amplitude data for each block of ten trials (five blocks/test session) were analyzed using a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group-by-block interaction, the block values were analyzed using Dunnett's test to determine if the treated group was significantly different from the control.

   Passive avoidance latency data were analyzed using a Wilcoxon Test for time to failure. The number of trials to criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats not meeting the criterion level in the learning phase was analyzed as incidence data.

   Water maze latency data were analyzed by a univariate ANOVA, with *post-hoc* analysis using Dunnett's test. The number of trials to criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats not meeting the criterion level in the learning phase was analyzed as incidence data.

   Pathology data were screened for potential effects and then evaluated using the table below:

Data Type	Data	Statistical Tests
	Organ weight	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Gross brain measurements	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Microscopic brain measurements	ANOVA and/or t-test (2)
Frequency	Ophthalmology	Visually Screened (3)
	Gross pathology	Visually Screened (3)
	Micropathology	Chi-Square Fisher's Exact Test

(1) ANOVA was used if data were homogeneous; Kruskal-Wallis was used if data were nonhomogeneous.
(2) A t-test, 2-tailed, was used for two-group comparisons; an ANOVA was used for multiple-group comparisons.
(3) If potential compound effects were suspected, then a Chi-square and one-tailed Fisher's Exact test were used.
All statistical tests used a significance level of $p \leq 0.05$, except for Bartlett's test, which used $p \leq 0.01$.

2. **Indices:**

a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Mating index (\%)} = \frac{\text{number of inseminated females}}{\text{number of females cohoused with males}} \times 100$$

$$\text{Female fertility index (\%)} = \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

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

$$\text{Viability index (\%)} = \frac{\text{number of live pups on day 4 preculling per litter}}{\text{number of live pups born per litter}} \times 100$$

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

3. **Positive and historical control data:** References were made to positive control studies for neurobehavioral testing (motor activity, acoustic startle habituation, passive avoidance and water maze), but no actual data were presented. **The only historical control data submitted with the study were brain morphometric measurements.** These submitted historical control data were not adequately described.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No parental females died or were sacrificed moribund during the study. No treatment-related clinical signs or changes in the FOB testing were observed in females.

2. **Body weight and food consumption**: Selected group mean body weight, body weight gain and food consumption values for pregnant and nursing dams are summarized in Table 2. However, summary data for 7 dams during lactation was omitted. It appears from the individual animal data that these 7 dams underwent "elective sacrifice". No further explanation was provided. Mean body weight during gestation was unaffected by treatment. However, at 4700 ppm mean body weight gain was decreased (8% of control value) during GDs 6-20 due to body weight gain (27% of control level) during GDs 6-13. Food consumption during GDs 6-13 was decreased (7% of control value) at 1000 ppm. The significant increase in food consumption during this period by females at 4700 ppm was not considered accurate since food spillage occurred. For subsequent weeks, grates were placed in the feeders of high-dose animals to reduce spillage. Mean body weight during lactation was slightly decreased (3-5% of control value) in females at 4700 ppm. Food consumption during lactation was unaffected by treatment.

TABLE 2. Selected mean (±SD) maternal body weight, body weight gain and food consumption[a]					
Observations/study interval	Dietary concentration (ppm)				
	0	60	250	1000	4700
Gestation (n=29-30)					
Body wt. GD 0 (g)	197.3±2.6	198.2±2.2	198.7±2.2	191.9±2.6	197.6±1.8
Body wt. GD 13 (g)	244.3±3.0	244.1±2.2	246.1±2.3	236.5±3.1	237.6±2.5
Body wt. GD 20 (g)	303.8±3.9	302.7±3.9	308.7±3.2	294.7±4.4	296.2±2.8
Wt. gain GDs 6-13 (g)[b]	25	23.4	27.1	22.6 (10)[c]	18.2 (27)
Wt. gain GDs 6-20 (g)	106.6±2.0	104.6±2.5	110.0±2.7	102.8±2.4 (4)	98.6*±1.9 (8)
Food cons. GDs 6-13 (g/day)	18.4±0.3	18.7±0.4	18.5±0.4	17.1*±0.3 (7)	30.3**±2.4 (65)
Food cons. GDs 13-20 (g/day)	20.7±0.4	21.3±0.5	21.2±0.5	19.7±0.5	20.7±0.6
Lactation (n=23-30)					
Body wt. LD 0 (g)	233.7±2.7	237.3±2.8	237.4±3.4	226.7±2.9	225.0±2.9 (4)
Body wt. LD 14 (g)	273.3±4.5	278.3±3.6	277.2±3.3	271.6±4.0	260.3±2.8 (5)
Body wt. LD 21 (g)	264.9±4.4	270.3±3.4	270.4±3.7	265.1±4.0	256.9±2.9 (3)
Wt gain LDs 0-21(g)[b]	31.2	33.0	33.0	38.4	31.9
Food cons. LDs 0-7 (g/day)	34.6±1.0	35.4±1.6	37.3±2.6	41.5±3.8	33.8±1.0
Food cons. LDs 7-14 (g/day)	51.4±1.1	51.8±1.3	52.3±0.9	50.7±1.1	49.3±1.4
Food cons. LDs 14-21 (g/day)	62.9±1.0	65.9±2.5	68.4±2.8	63.5±2.4	60.8±1.5

[a]Data obtained from pages 55, 57, 62 and 64, MRID 46590001.
[b]Calculated by the reviewer without standard deviations.
[c]Number in parentheses is percent change, relative to control; calculated by reviewer.
GD = Gestation Day; LD = Lactation Day; Food cons. = Food consumption.
* Significantly different from control value, $p<0.05$

3. **Test substance intake:** Based on maternal food consumption and body weight and nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3.

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day)[a]				
Period	60 ppm	250 ppm	1000 ppm	4700 ppm
Gestation				
Days 6-13	5.4	23.0	83.3	685[b]
Days 13-20	5.5	23.4	87.1	433
Lactation				
Days 0-7	5.1	22.9	106.3	412
Days 7-14	5.6	23.3	94.1	442
Days 14-21	5.7	23.5	90.7	430
Average (Gestation and Lactation)	5.5	23.2	92.3	429.3

[a]Data obtained from page 39, MRID 46590001. Dietary concentrations were reduced to 0/0/0, 34/28/24, 145/116/95, 584/471/388, and 2716/2222/1844 ppm during weeks 1/2/3 of lactation, respectively, based on estimated increases in feed consumption (g consumed/kg body weight/day) during lactation.
[b]Associated with observed food spillage and considered an unreliable measure of active ingredient intake. This value was excluded from the mean average daily intake.

4. **Reproductive performance**: The fertility index was 100% for the control, 60 and 4700 ppm groups and 96.7% for the 250 and 1000 ppm groups. The mean duration of gestation was unaffected by treatment. Results for the maternal animals are summarized in Table 4.

TABLE 4. Reproductive performance[a]

Observation	Dietary concentration (ppm)				
	0	60	250	1000	4700
Number co-housed	30	30	30	30	30
Mating index (%)	100	100	100	100	100
Fertility index (%)	100	100	96.7	96.7	100
Intercurrent deaths	0	0	0	0	0
Mean (±SE) gestation duration (days)	21.6 ± 0.1	21.6 ± 0.1	21.7 ± 0.1	21.8 ± 0.1	21.7 ± 0.1

[a] Data obtained from page 51, MRID 46590001.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 5. No treatment-related effects were observed on reported litter size, survival or clinical signs. However, data from 7 litters were not reported. Given that the fertility index was approximately 100% for all doses, this omission is inexplicable. It appears from the individual animal data, however, that the size of these 7 litters was in many cases much smaller than the mean. One control male pup was found dead on PND 59, the day after the learning phase of water maze testing. At necropsy, water was found in the animal's lungs so the death was attributed to the water maze testing. Two low-dose pups were found dead on PND 7 and 9 (1 cannibalized). Three pups at 1000 ppm were either found dead on PND 6 or were missing on PND 5. Two animals at 1000 ppm were sacrificed at the request of the study director on PND 28 or PND 48 due to exophthalmia. Two pups at 4700 ppm were missing on PND 4 after culling or on PND 11. The deaths were not considered treatment-related.

   Clinical signs observed in both control and treated groups during lactation and post-weaning included bruising at various locations, urine stain, red lacrimal stain, red nasal stain, areas of alopecia and minor dermal and ocular lesions.

TABLE 5. Litter size and viability of F_1 rats [a]

Observation	Dietary concentration (ppm)				
	0	60	250	1000	4700
No. of litters [b]	23	23	23	23	23
Total number of pups born	255	254	265	256	264
Total no. of pups missing	1	2	0	2	2
Litters with pups missing	1	2	0	1	2
Total no. of pups found dead	0	1	0	4	0
Litters with pups found dead	0	1	0	2	0
Total no. of pups cannibalized	0	1	0	0	0
Litters with pups cannibalized	0	1	0	0	0
Litter size (mean ± S.E.)	11.1 ± 0.3	11.0 ± 0.5	11.5 ± 0.3	11.1± 0.4	11.5 ± 0.3
No. of stillborn pups	0	1	1	0	0
Mean no. of viable pups [c]					
Birth	11	11	11	11	11
Day 4 (precull)	11	11	11	11	11
Day 4 (postcull)	8	8	8	8	8
Day 21	8	8	8	8	8
Live Birth Index (±S.E.)	100.0 ± 0.0	99.6 ± 0.4	99.7 ± 0.3	100.0 ± 0.0	100.0 ± 0.0
Viability Index (±S.E.)	99.6 ± 0.4	99.3 ± 0.5	100.0 ± 0.0	99.0 ± 1.0	99.6 ± 0.4
Lactation Index (±S.E.)	100.0 ± 0.0	98.9 ± 0.8	100.0 ± 0.0	98.4 ± 1.2	99.5 ± 0.5

[a] Data obtained from pages 69-70, MRID 46590001.
[b] Data from 7 litters were not reported. Given that the fertility index was approximately 100% for all doses, this omission is inexplicable. It appears from the individual animal data, however, that the size of these 7 litters was in many cases much smaller than the mean.
[c] Calculated as the sum of the mean number of live pups/sex and then rounded to the nearest whole number.

2. **Body weight:** Selected mean preweaning pup body weight and body weight gain data are presented in Table 6. No treatment-related effects were observed at birth; however, body weight was significantly decreased in males (9-10%, relative to control values) and females (8-11%, relative to control values) at 4700 ppm from PNDs 4-21. Body weight gain during the pre-weaning period was significantly decreased in males (9-20%, relative to controls) and females (8-20%, relative to controls) at 4700 ppm.

   Post-weaning (PNDs 29-72) body weight was significantly decreased in males (3-6%, relative to controls) and females (4-5%, relative to controls) at 4700 ppm during the first three weeks post-weaning. Body weight gain was not affected by treatment. Selected mean post-weaning pup body weight and body weight gain data are presented in Table 7.

TABLE 6. Selected mean (±SE) pre-weaning pup body weight and body weight gain [a]

PND	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
Body Weight (g)					
0	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	6.0 ± 0.1	5.7 ± 0.1
4 (pre-cull)	9.9 ± 0.2	9.6 ± 0.2	9.8 ± 0.2	10.1 ± 0.3	9.0* ± 0.3 (9)[b]
4 (post-cull)	9.9 ± 0.2	9.7 ± 0.2	9.8 ± 0.2	10.1 ± 0.3	9.0* ± 0.3 (9)
11	25.0 ± 0.6	24.8 ± 0.6	25.5 ± 0.4	25.3 ± 0.6	22.6** ± 0.5 (10)
17	38.0 ± 0.7	38.3 ± 0.7	38.9 ± 0.4	38.6 ± 0.8	34.6** ± 0.6 (9)
21	48.6 ± 0.8	48.8 ± 1.0	49.2 ± 0.6	50.0 ± 1.1	44.4** ± 0.9 (9)
Body Weight Gain (g)					
0-4	4.1 ± 0.2	3.8 ± 0.2	4.0 ± 0.2	4.1 ± 0.2	3.3** ± 0.2 (20)
4-11	15.1 ± 0.4	15.1 ± 0.4	15.7 ± 0.2	15.3 ± 0.5	13.6* ± 0.3 (10)
4-17	28.1 ± 0.6	28.6 ± 0.6	29.1 ± 0.3	28.5 ± 0.6	25.6** ± 0.5 (9)
4-21	38.7 ± 0.7	39.1 ± 0.8	39.4 ± 0.4	39.9 ± 0.9	35.4** ± 0.7 (9)
Females					
Body Weight (g)					
0	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.4 ± 0.1
4 (pre-cull)	9.6 ± 0.2	9.5 ± 0.2	9.5 ± 0.2	9.7 ± 0.3	8.7* ± 0.3 (9)
4 (post-cull)	9.7 ± 0.2	9.4 ± 0.2	9.5 ± 0.2	9.7 ± 0.3	8.7* ± 0.3 (10)
11	24.5 ± 0.6	24.4 ± 0.5	24.9 ± 0.4	24.6 ± 0.6	22.3* ± 0.4 (9)
17	37.8 ± 0.7	37.3 ± 0.7	37.7 ± 0.5	37.1 ± 0.8	33.8** ± 0.5 (11)
21	47.5 ± 0.9	47.4 ± 0.8	47.8 ± 0.6	47.9 ± 1.0	43.7** ± 0.7 (8)
Body Weight Gain (g)					
0-4	4.1 ± 0.2	3.9 ± 0.2	4.0 ± 0.1	4.0 ± 0.2	3.3** ± 0.2 (20)
4-11	14.8 ± 0.5	15.0 ± 0.3	15.4 ± 0.3	14.9 ± 0.4	13.5 ± 0.2 (9)
4-17	28.1 ± 0.6	27.9 ± 0.6	28.3 ± 0.4	27.4 ± 0.6	25.1** ± 0.4 (11)
4-21	37.8 ± 0.7	37.9 ± 0.6	38.4 ± 0.5	38.1 ± 0.8	34.9** ± 0.5 (8)

[a] Data obtained from pages 78-83, MRID 46590001.
[b] Number in parentheses is percent change, relative to control; calculated by reviewer.
PND = post-natal day
N = 23
* Statistically significantly different from control, $p \leq 0.05$
** Statistically significantly different from control, $p \leq 0.01$

TABLE 8. Selected mean (±SD) post-weaning pup body weight and body weight gain [a]					
PND	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
Body weight (g)					
29	78.6 ± 6.7	79.2 ± 8.2	80.1 ± 6.5	80.7 ± 7.2	73.7* ± 7.5 (6)[b]
36	123.2 ± 9.1	125.4 ± 11.5	126.1 ± 10.2	127.3 ± 10.2	117.3* ± 16.1 (5)
50	209.3 ± 13.5	213.5 ± 18.6	214.6 ± 16.8	213.1 ± 16.8	203.6 ± 20.9 (3)
71	313.3 ± 20.1	319.1 ± 26.6	319.5 ± 24.5	316.4 ± 26.1	307.8 ± 27.9
Body weight Gain (g)[c]					
29-36	44.6	46.2	46.0	46.6	43.6
36-71	190.1	193.7	193.4	189.1	190.5
29-71	234.7	239.9	239.4	235.7	234.1
Females					
Body weight (g)					
30	76.5 ± 5.9	76.7 ± 6.7	77.7 ± 5.8	77.3 ± 7.6	72.5* ± 7.8 (5)
37	112.5 ± 6.8	112.9 ± 9.8	113.0 ± 7.7	112.9 ± 9.6	108.2* ± 8.5 (4)
51	151.5 ± 10.8	154.0 ± 12.3	153.2 ± 10.0	151.2 ± 13.0	148.1 ± 11.6
72	191.2 ± 13.4	193.9 ± 16.5	192.1 ± 12.7	192.0 ± 15.8	190.0 ± 15.5
Body weight Gain (g)[c]					
30-37	36.0	36.2	35.3	35.6	35.7
37-72	78.7	81.0	79.1	79.1	81.8
30-72	114.7	117.2	114.4	114.7	117.5

[a] Data obtained from pages 89-90, MRID 46590001.
[b] Number in parentheses is percent change, relative to control; calculated by reviewer.
[c] Calculated by the reviewer without standard deviations.
PND = post-natal day
* Statistically significantly different from control, $p \leq 0.05$

3. Developmental landmarks:

a. **Sexual maturation:** These data are presented in Table 9. The average age of onset of preputial separation in males was 43.3, 43.6, 43.1, 43.3 and 44.4 days for the control, 60, 250, 1000 and 4700 ppm groups, respectively. The average age of onset of vaginal opening was 33.2, 33.8, 32.8, 32.9 and 32.6 days for the control, 60, 250, 1000 and 4700 ppm groups, respectively. Body weight at attainment for males and females was not reported, although the protocol indicated that weight should be measured.

b. **Other developmental landmarks:** Pupil constriction in response to a penlight was manifest in both control and treated groups by PND 21. The data are presented in Table 9.

TABLE 9. Attainment of developmental landmarks (mean ±SE) [a]					
Parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
N (M/F)	23/23	23/23	23/23	23/23	23/23
Preputial separation Mean age (days)	43.3 ± 0.4	43.6 ± 0.3	43.1 ± 0.3	43.3 ± 0.3	44.4 ± 0.5
Vaginal opening Mean age (days)	33.2 ± 0.5	33.8 ± 0.6	32.8 ± 0.4	32.9 ± 0.3	32.6 ± 0.3
Pupil Constriction Pups reaching criterion by PND 21 (%)	100	100	100	100	100

[a] Data obtained from page 87, MRID 46590001.

4. Behavioral assessment:

a. **Functional observational battery:** One male from the 1000 ppm group that was assigned to this test was found dead on PND 7 and therefore was not tested after PND 4. Another male at 1000 ppm was sacrificed due to exopthalmia on PND 49 and therefore, was not tested on PND 60. As a result, the group sizes through PND 60 were 14-16/sex/group, representing a minimum of 20 liters/group. No treatment-related findings were observed. Incidental findings included urine stain on PND 35 in one male at 4700 ppm, exopthalmia observed on or after PND 35 (one male each at 60, 1000, and 4700 ppm and one female at 60 ppm) and external lesions (scabs) in five males and one female in various dose groups and controls on PND 60.

b. **Motor/locomotor activity:** Total motor and locomotor activity data for PNDs 13, 17, 21 and 60 are presented in Table 10. Sub-session (interval) data for motor and locomotor activity are included in Tables 11 and 12, respectively. One female from the 1000 ppm group that was allocated for this test was sacrificed due to marked unilateral exophthalmia on PND 29 and therefore, was not tested on PND 60. Thus, the group sizes through PND 60 were 15-16/sex/group. There was no evidence of a treatment-related effect. An apparent increase in mean overall motor activity was observed at PNDs 13 and 17; however, variability around the means was high, and the increase was not statistically significant. The only statistical difference from control was a significantly lower level of locomotor activity for females at 250 ppm during the second interval of PND 21. Motor and locomotor activity habituation was evident in both sexes at all ages tested, except on PND 13 when mean locomotor activity levels were so low (1-3 movements/interval) during the first intervals that habituation was not evident.

TABLE 10. Mean (±S.D.) motor and locomotor activity data (total activity counts for session) in F_1 rats [a]

PND	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
Motor activity					
13	71 ± 67	72 ± 52	50 ± 49	102 ± 100	107 ± 78
17	139 ± 113	153 ± 117	189 ± 114	185 ± 139	186 ± 96
21	262 ± 97	344 ± 83	315 ± 86	267 ± 68	289 ± 126
60	507 ± 101	539 ± 137	502 ± 104	506 ± 99	478 ± 93
Locomotor activity					
13	10 ± 18	11 ± 13	8 ± 16	16 ± 21	8 ± 11
17	34 ± 35	32 ± 35	42 ± 27	45 ± 42	44 ± 29
21	78 ± 33	103 ± 30	95 ± 35	91 ± 30	87 ± 36
60	360 ± 93	368 ± 115	346 ± 81	342 ± 98	318 ± 70
Females					
Motor activity					
13	67 ± 50	71 ± 65	81 ± 74	96 ± 143	80 ± 64
17	138 ± 88	157 ± 92	115 ± 76	170 ± 123	173 ± 107
21	343 ± 171	332 ± 124	254 ± 90	330 ± 109	295 ± 97
60	800 ± 203	741 ± 229	761 ± 141	756 ± 190	659 ± 115
Locomotor activity					
13	4 ± 6	9 ± 12	13 ± 16	13 ± 36	9 ± 11
17	36 ± 29	37 ± 27	26 ± 20	43 ± 31	52 ± 36
21	98 ± 38	91 ± 33	75 ± 32	103 ± 44	82 ± 22
60	515 ± 134	487 ± 137	488 ± 86	493 ± 159	418 ± 89

[a] Data obtained from pages 174-178, MRID 46590001.
PND = post-natal day
N=15-16

TABLE 11: Mean (± SD) sub-session motor activity (# movements/10-minute interval) in F_1 rats[a]

Interval	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
PND 13					
1	16 ± 12	19 ± 20	16 ± 13	35 ± 28	24 ± 18
2	15 ± 26	13 ± 17	9 ± 7	22 ± 26	18 ± 20
3	14 ± 21	11 ± 12	9 ± 16	22 ± 42	14 ± 12
4	13 ± 18	7 ± 10	6 ± 12	12 ± 19	16 ± 32
5	7 ± 9	5 ± 10	5 ± 11	7 ± 15	22 ± 27
6	6 ± 9	18 ± 32	6 ± 17	4 ± 6	13 ± 32
PND 17					
1	55 ± 46	54 ± 31	79 ± 49	58 ± 35	60 ± 35
2	22 ± 30	27 ± 31	39 ± 28	37 ± 32	39 ± 23
3	25 ± 29	9 ± 21	22 ± 21	27 ± 30	27 ± 24
4	11 ± 19	20 ± 27	17 ± 27	25 ± 32	31 ± 30
5	13 ± 22	15 ± 31	14 ± 17	23 ± 34	16 ± 23
6	12 ± 19	26 ± 38	18 ± 26	16 ± 22	14 ± 21
PND 21					
1	112 ± 30	123 ± 23	116 ± 23	110 ± 24	105 ± 34
2	60 ± 30	73 ± 23	70 ± 20	63 ± 22	55 ± 24
3	34 ± 24	64 ± 29	55 ± 25	45 ± 24	43 ± 21
4	23 ± 27	36 ± 32	31 ± 23	32 ± 21	37 ± 27
5	17 ± 21	30 ± 21	23 ± 28	11 ± 16	22 ± 28
6	16 ± 15	18 ± 19	21 ± 23	6 ± 14	28 ± 31
PND 60					
1	104 ± 22	109 ± 22	105 ± 26	104 ± 18	106 ± 14
2	94 ± 29	100 ± 40	85 ± 29	85 ± 22	91 ± 28
3	84 ± 24	90 ± 33	94 ± 31	87 ± 31	84 ± 22
4	80 ± 28	92 ± 25	82 ± 28	83 ± 25	75 ± 22
5	79 ± 24	85 ± 25	82 ± 24	78 ± 32	72 ± 19
6	66 ± 20	64 ± 27	52 ± 23	69 ± 25	51 ± 23
Females					
PND 13					
1	16 ± 12	27 ± 24	13 ± 18	22 ± 23	20 ± 13
2	16 ± 18	19 ± 30	21 ± 25	25 ± 38	15 ± 24
3	11 ± 20	5 ± 9	14 ± 20	24 ± 43	13 ± 20
4	8 ± 20	7 ± 16	8 ± 17	6 ± 10	5 ± 8
5	7 ± 10	4 ± 6	10 ± 16	12 ± 32	15 ± 27
6	8 ± 16	9 ± 13	15 ± 19	7 ± 16	12 ± 17

Interval	Dietary concentration (ppm)				
	0	60	250	1000	4700
PND 17					
1	50 ± 37	65 ± 32	49 ± 34	64 ± 34	53 ± 35
2	32 ± 28	33 ± 25	25 ± 23	41 ± 31	35 ± 22
3	16 ± 17	21 ± 23	17 ± 23	25 ± 27	26 ± 26
4	14 ± 18	11 ± 15	4 ± 12	18 ± 26	17 ± 20
5	14 ± 25	15 ± 20	9 ± 18	10 ± 16	22 ± 33
6	11 ± 25	11 ± 19	11 ± 18	12 ± 17	18 ± 28
PND 21					
1	125 ± 36	116 ± 30	106 ± 23	114 ± 18	108 ± 22
2	78 ± 41	71 ± 35	46 ± 19	67 ± 22	61 ± 28
3	47 ± 37	53 ± 18	41 ± 29	59 ± 36	53 ± 33
4	34 ± 25	41 ± 26	29 ± 25	43 ± 21	29 ± 26
5	28 ± 35	35 ± 33	17 ± 22	30 ± 33	20 ± 21
6	31 ± 30	17 ± 24	16 ± 24	16 ± 23	24 ± 25
PND 60					
1	140 ± 35	135 ± 43	139 ± 34	139 ± 39	126 ± 31
2	129 ± 47	113 ± 44	129 ± 35	129 ± 49	111 ± 30
3	142 ± 42	131 ± 52	131 ± 29	130 ± 38	117 ± 43
4	138 ± 41	120 ± 39	138 ± 37	129 ± 48	112 ± 27
5	141 ± 41	122 ± 49	119 ± 43	121 ± 47	107 ± 37
6	110 ± 33	121 ± 61	105 ± 26	109 ± 35	87 ± 27

[a] Data were obtained from pages 180-187, MRID 46590001.
PND = postnatal day
N = 15-16

TABle 12: Mean (± SD) sub-session locomotor activity (# movements/10 minute interval) in F_1 rats[a]

Interval	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
PND 13					
1	3 ± 3	3 ± 5	4 ± 4	4 ± 4	1 ± 1
2	2 ± 5	1 ± 1	1 ± 1	4 ± 6	1 ± 3
3	2 ± 5	2 ± 3	1 ± 2	4 ± 9	1 ± 1
4	2 ± 5	1 ± 2	1 ± 2	2 ± 5	2 ± 4
5	1 ± 2	1 ± 2	1 ± 3	2 ± 4	2 ± 4
6	1 ± 1	4 ± 10	2 ± 7	0 ± 1	1 ± 4
PND 17					
1	13 ± 13	10 ± 9	17 ± 12	17 ± 15	14 ± 10
2	5 ± 6	6 ± 8	8 ± 8	8 ± 9	9 ± 7
3	7 ± 9	2 ± 7	4 ± 5	6 ± 8	7 ± 8
4	3 ± 6	5 ± 8	4 ± 7	6 ± 7	6 ± 9
5	3 ± 8	3 ± 7	4 ± 5	5 ± 8	5 ± 8
6	3 ± 5	6 ± 9	5 ± 7	4 ± 8	2 ± 4
PND 21					
1	39 ± 13	43 ± 11	40 ± 12	42 ± 8	37 ± 13
2	15 ± 8	19 ± 7	19 ± 7	20 ± 9	15 ± 7
3	10 ± 8	19 ± 10	16 ± 9	14 ± 10	12 ± 6
4	6 ± 8	8 ± 9	8 ± 8	10 ± 6	10 ± 7
5	5 ± 7	9 ± 7	6 ± 8	3 ± 5	6 ± 7
6	3 ± 5	5 ± 6	6 ± 7	1 ± 4	7 ± 8
PND 60					
1	81 ± 22	79 ± 21	78 ± 20	73 ± 15	71 ± 12
2	69 ± 28	67 ± 36	57 ± 23	57 ± 21	61 ± 22
3	58 ± 22	59 ± 28	66 ± 25	60 ± 29	57 ± 19
4	55 ± 23	65 ± 21	57 ± 22	57 ± 20	52 ± 19
5	54 ± 21	58 ± 23	53 ± 21	50 ± 25	45 ± 13
6	42 ± 16	40 ± 20	35 ± 19	46 ± 21	32 ± 20
Females					
PND 13					
1	1 ± 1	3 ± 4	3 ± 3	2 ± 3	3 ± 2
2	0 ± 1	3 ± 6	4 ± 6	4 ± 12	1 ± 3
3	0 ± 1	1 ± 2	2 ± 5	4 ± 10	2 ± 3
4	0 ± 1	1 ± 1	1 ± 2	0 ± 1	1 ± 1
5	0 ± 1	0 ± 1	1 ± 2	2 ± 6	2 ± 5
6	1 ± 4	1 ± 1	3 ± 6	1 ± 4	1 ± 2

Interval	Dietary concentration (ppm)				
	0	60	250	1000	4700
PND 17					
1	13 ± 13	15 ± 11	12 ± 11	16 ± 10	16 ± 12
2	7 ± 7	9 ± 8	4 ± 4	10 ± 8	9 ± 7
3	5 ± 6	4 ± 6	4 ± 5	6 ± 7	7 ± 8
4	3 ± 5	2 ± 4	1 ± 3	5 ± 7	7 ± 8
5	4 ± 7	3 ± 4	2 ± 6	2 ± 4	7 ± 10
6	4 ± 12	3 ± 6	4 ± 8	3 ± 5	5 ± 10
PND 21					
1	41 ± 9	39 ± 10	37 ± 11	41 ± 11	34 ± 7
2	22 ± 13	16 ± 8	11 ± 6 *	19 ± 10	15 ± 6
3	13 ± 9	15 ± 6	11 ± 8	16 ± 10	15 ± 8
4	8 ± 7	9 ± 6	8 ± 8	13 ± 6	7 ± 6
5	7 ± 8	9 ± 9	5 ± 7	9 ± 9	5 ± 5
6	7 ± 7	3 ± 5	4 ± 7	5 ± 7	7 ± 8
PND 60					
1	86 ± 18	84 ± 20	85 ± 16	90 ± 24	82 ± 22
2	78 ± 30	71 ± 26	78 ± 30	81 ± 37	67 ± 20
3	95 ± 34	87 ± 38	91 ± 28	87 ± 41	74 ± 32
4	95 ± 35	84 ± 30	92 ± 32	88 ± 40	73 ± 25
5	96 ± 28	79 ± 32	79 ± 30	77 ± 34	70 ± 32
6	66 ± 26	82 ± 42	63 ± 24	69 ± 31	52 ± 25

[a] Data were obtained from pages 189-196, MRID 4590001.
PND = postnatal day
N = 15-16
* Significantly different from control, $p \leq 0.05$

c. **Auditory startle reflex habituation:** The summary amplitude and latency data for PNDs 22 and 60 are presented in Table 13. Interval data are included in Table 14. No treatment-related changes in startle amplitude, latency, or habituation were observed. Statistical differences from control seen in males at both 250 and 1000 ppm on PND 22 included increased mean response amplitudes for the entire session and for the first (250 and 1000 ppm) and last two (1000 ppm) blocks of trials. Females at 250 ppm had a significantly higher average response amplitude for the entire session and for the first two blocks of trials on PND 60. These findings were not considered treatment-related due to a lack of consistent pattern across treatment groups. Habituation (decrease in mean response amplitude over the course of the test session) was apparent in control and treated animals on both testing days.

TABLE 13. Mean (±S.D.) auditory startle peak amplitude (g) and latency to peak (msec) in F_1 male and female rats [a]

Dietary concentration	Dosage (ppm)				
	0	60	250	1000	4700
Males					
PND 22					
Number of animals	16	16	16	16	16
Mean body weight [b]	52	52	54	55	50
Peak amplitude	22 ± 10	28 ± 14	34 ± 13 *	33 ± 15 *	24 ± 9
Latency to peak	38 ± 4	37 ± 4	36 ± 3	37 ± 5	41 ± 6
PND 60					
Number of animals	16	16	16	16	16
Mean body weight	264	272	275	274	271
Peak amplitude	153 ± 146	154 ± 105	162 ± 104	188 ± 118	184 ± 96
Latency to peak	41 ± 3	40 ± 3	41 ± 3	40 ± 2	40 ± 2
Females					
PND 22					
Number of animals	16	16	16	16	16
Mean body weight	51	52	51	52	46
Peak amplitude	26 ± 10	23 ± 12	26 ± 10	28 ± 11	22 ± 8
Latency to peak	37 ± 4	38 ± 4	39 ± 5	38 ± 3	42 ± 7
PND 60					
Number of animals	16	16	16	16	16
Mean body weight	174	175	170	176	171
Peak amplitude	72 ± 57	77 ± 44	127 ± 71 *	97 ± 67	86 ± 52
Latency to peak	41 ± 3	41 ± 4	41 ± 5	42 ± 3	42 ± 3

[a]Data obtained from 198-199, MRID 46590001
[b]Based on the mean output for the first 8 msec (baseline) of all 50 trials
PND = postnatal day
Peak amplitude = mean ± S.D., measured in grams
Latency to peak = mean ± S.D., measured in milliseconds
* Significantly different from control, $p \leq 0.05$

TABLE 14: Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 male and female rats [a]

Conc. (ppm)	Parameter	Trials 1-10	Trials 11-20	Trials 21-30	Trials 31-40	Trials 41-50
Males - PND 22						
0	Peak amplitude	23 ± 8	25 ± 14	23 ± 13	20 ± 11	17 ± 9
	Latency to peak	37 ± 4	35 ± 3	37 ± 5	39 ± 7	41 ± 7
60	Peak amplitude	31 ± 13	29 ± 17	30 ± 15	26 ± 12	27 ± 15
	Latency to peak	37 ± 3	38 ± 6	37 ± 6	37 ± 6	36 ± 4
250	Peak amplitude	41 ± 15 *	35 ± 15	35 ± 15	31 ± 15	27 ± 13
	Latency to peak	37 ± 4	37 ± 7	34 ± 3	37 ± 5	37 ± 3
1000	Peak amplitude	37 ± 14 *	33 ± 15	35 ± 17	32 ± 16 *	30 ± 15 *
	Latency to peak	38 ± 4	37 ± 5	37 ± 6	38 ± 8	37 ± 5
4700	Peak amplitude	27 ± 9	26 ± 15	24 ± 10	21 ± 9	20 ± 7
	Latency to peak	43 ± 8	41 ± 8	40 ± 7	42 ± 9	39 ± 7
Males - PND 60						
0	Peak amplitude	186 ± 137	187 ± 204	146 ± 184	130 ± 123	116 ± 110
	Latency to peak	42 ± 3	40 ± 3	41 ± 3	42 ± 7	41 ± 6
60	Peak amplitude	189 ± 122	170 ± 144	143 ± 97	139 ± 95	130 ± 94
	Latency to peak	42 ± 3	41 ± 4	40 ± 4	38 ± 5	39 ± 4
250	Peak amplitude	201± 134	197 ± 122	165 ± 119	131 ± 104	114 ± 90
	Latency to peak	42 ± 3	40 ± 4	41 ± 4	40 ± 4	41 ± 4
1000	Peak amplitude	229 ± 119	214 ± 130	196 ± 143	161 ± 129	138 ± 112
	Latency to peak	43 ± 3	40 ± 2	40 ± 4	39 ± 3	39 ± 3
4700	Peak amplitude	225 ± 132	233 ± 102	193 ± 135	151 ± 110	117 ± 72
	Latency to peak	43 ± 2	41±2	40±3	39±3	40±4
Females - PND 22						
0	Peak amplitude	29 ± 12	28 ± 13	24 ± 11	26 ± 11	24 ± 11
	Latency to peak	40 ± 6	37 ± 6	36 ± 5	35 ± 5	37 ± 4
60	Peak amplitude	25 ± 13	25 ± 13	25 ± 13	21 ± 12	19 ± 11
	Latency to peak	41 ± 6	38 ± 6	37 ± 7	38 ± 3	37 ± 4
250	Peak amplitude	27 ± 11	29 ± 11	27 ± 13	25 ± 12	24 ± 9
	Latency to peak	40 ± 5	41 ± 7	38 ± 6	38 ± 6	38 ± 5
1000	Peak amplitude	33 ± 16	29 ± 14	27 ± 10	26 ± 12	26 ± 11
	Latency to peak	38 ± 4	36 ± 3	37 ± 7	36 ± 3	40 ± 7
4700	Peak amplitude	24 ± 10	21 ± 8	22 ± 11	23 ± 11	22 ± 9
	Latency to peak	43 ± 6	44 ± 9	40 ± 8	41 ± 9	43 ± 8

Conc. (ppm)	Parameter	Trials 1-10	Trials 11-20	Trials 21-30	Trials 31-40	Trials 41-50
Females - PND 60						
0	Peak amplitude	90 ± 78	73 ± 65	76 ± 64	63 ± 47	56 ± 51
	Latency to peak	43 ± 5	41 ± 5	40 ± 6	39 ± 5	41 ± 5
60	Peak amplitude	92 ± 63	88 ± 53	78 ± 52	69 ± 55	57 ± 28
	Latency to peak	42 ± 5	42 ± 4	40 ± 5	39 ± 4	41 ± 5
250	Peak amplitude	164 ± 78 *	157 ± 113 *	129 ± 100	98 ± 57	85 ± 50
	Latency to peak	43 ± 6	42 ± 6	40 ± 5	42 ± 6	41 ± 5
1000	Peak amplitude	119 ± 68	122 ± 103	107 ± 88	77 ± 64	61 ± 40
	Latency to peak	42 ± 4	42 ± 5	41 ± 4	41 ± 4	41 ± 6
4700	Peak amplitude	94 ± 51	94 ± 65	94 ± 83	83 ± 48	64 ± 45
	Latency to peak	44 ± 3	43 ± 4	40 ± 5	40 ± 4	41 ± 5

[a] Data obtained from pages 201-204, MRID 46590001.
N = 15-16
PND = postnatal day
Peak amplitude = mean ± S.D., measured in grams
Latency to peak = mean ± S.D., measured in milliseconds
* Significantly different from control, $p \leq 0.05$

d. Learning and memory testing:

1) **Passive avoidance testing:** The passive avoidance performance data for PNDs 22 and 29 are summarized in Table 15. On the first test, acquisition was evident in control males and females as a marked increase in the latency to cross for the second trial, compared to the first trial. On the second test, retention was evident in control males and females as a protracted delay to cross within the 180-second time limit of the first trial, compared to the first test day. No treatment-related effects were observed. The only significant change was an increase in latency for the first trial of the learning phase in females at 1000 ppm.

TABLE 15. Passive avoidance performance data (mean ±SD) in F_1 rats [a]

Parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
Session 1 (PND 22) [b]					
Number animals tested	16	16	16	16	16
Number of animals in analysis	16	16	16	16	16
Trials to criterion	2.9±0.3	3.1±0.3	3.1±0.3	3.0±0.0	2.9±0.3
Latency trial 1 (sec)	42.6±41.1	27.8±23.8	46.7±26.1	29.3±20.0	45.5±45.5
Latency trial 2 (sec)	180.0±0.0	174.1±19.3	164.7±44.4	180.0±0.0	180.0±0.0
No. failed to meet criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No. failed to cross during learning phase	1 (6%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)
Session 2 (PND 29) [b]					
Number of animals tested	15	16	16	16	16
Number of animals in analysis	15	16	16	16	15
Trials to criterion	2.1±0.3	2.1±0.5	2.3±0.7	2.3±0.7	2.0±0.0
Latency trial 1 (secs)	176.0±15.5	180.0±0.0	180.0±0.0	179.1±3.6	180.0±0.0
Latency trial 2 (secs)	180.0±0.0	173.3±26.8	177.9±6.8	169.4±29.4	180.0±0.0
Females					
Session 1 (PND 22) [b]					
Number animals tested	16	16	16	16	16
Number of animals in analysis	16	16	16	16	16
Trials to criterion	3.1±0.6	3.1±0.5	3.1±0.3	3.1±0.6	3.0±0.0
Latency trial 1 (secs)	30.8±45.2	32.9±23.1	36.2±27.4	54.3±45.0 *	24.1±16.9
Latency trial 2 (secs)	180.0±0.0	180.0±0.0	175.2±19.2	175.4±18.3	180.0±0.0
No. failed to meet criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No. failed to cross during learning phase	1 (6%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)
Session 2 (PND 29) [b]					
Number of animals tested	15	16	16	15	16
Number of animals in analysis	15	16	16	15	16
Trials to criterion	2.3±0.7	2.2±0.5	2.2±0.5	2.2±0.6	2.1±0.3
Latency trial 1 (secs)	176.1±15.2	179.6±1.7	174.9±20.5	177.5±9.7	166.3±39.8
Latency trial 2 (secs)	174.6±16.9	179.4±2.6	174.5±22.2	171.2±34.0	180.0±0.0

[a] Data obtained from pp. 206-207, MRID 46590001.
[b] Session 1= learning phase; Session 2 = retention phase
PND = postnatal day
Trials to criterion = mean number of trials per group ± S.D.
Latency to trial 1 = mean trial 1 duration (seconds) per group ± S.D.
Latency to trial 2 = mean trial 2 duration (seconds) per group ± S.D.
Failed to meet criterion = number of animals that received shock but did not demonstrate acquisition.
Failed to cross = number of animals that never received the shock, because failed to cross.

2) **Watermaze performance**: The watermaze performance data for PNDs 60±1 and 67±2 are summarized in Table 16. One control male assigned to this test was found dead on PND 59, one day after the learning phase of the testing. At necropsy, water was found in the lungs; therefore, the death was attributed to complications from the testing. One male at 1000 ppm was sacrificed due to exophthalmia on PND 49. As a result of these deaths, the number of animals tested was 15-16/sex/group. Because of the large variation surrounding the means for males and females (CV = 80-90%) for time to escape in Session 1/trial 2, acquisition was not evident after trial 2. However, although not tabulated, it was reported that the mean time to escape was 5.1 sec and 6.3 sec by trial 5 for control males and females, respectively. While examination of the individual animal

data supported this, the registrant needs to submit summary data for the other trials in each session to confirm the lack of effect on acquisition. Although not analyzed statistically, the mean times to escape for trials 1 and 2 in Session 2 (retention) were decreased relative to the mean times for trials 1 and 2 in Session 1. No treatment-related changes in average time to escape or number of trials-to-criterion were observed.

TABLE 16. Watermaze performance data (mean ±SD) in F_1 rats [a]

Parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
Session 1 (PND 60±2) [b]					
Number of animals	16	16	16	15	16
Trials to criterion	6.8 ± 1.4	6.9 ± 2.4	7.2 ± 3.3	7.0 ± 2.3	7.4 ± 2.3
Trial 1- errors	0.6 ± 0.7	0.6 ± 0.7	0.9 ± 1.1	0.9 ± 1.0	0.9 ± 0.8
Trial 1 - duration (secs)	12.1 ± 9.7	13.6 ± 8.1	18.7 ± 17.1	16.5 ± 14.7	19.3 ± 15.1
Trial 2 - errors	0.5 ± 0.9	0.5 ± 0.7	0.5 ± 1.3	0.4 ± 0.8	0.6 ± 0.8
Trial 2 - duration (secs)	11.3 ± 10.2	15.4 ± 16.4	14.7 ± 14.9	13.3 ± 10.7	14.6 ± 11.2
Failed to meet criterion	0 (0%)	0 (0%)	2 (13%)	0 (0%)	0 (0%)
Session 2 (PND 67±2) [b]					
Number of animals	15	16	14	15	16
Trials to criterion	6.1 ± 2.5	5.5 ± 0.7	5.6 ± 1.0	5.2 ± 0.6	5.3 ± 0.6
Trial 1 - errors	0.5 ± 0.8	0.4 ± 0.9	0.6 ± 1.0	0.1 ± 0.5	0.3 ± 0.6
Trial 1 - duration (secs)	8.9 ± 6.2	7.7 ± 5.0	13.5 ± 15.3	6.3 ± 4.8	8.3 ± 5.8
Trial 2 - errors	0.2 ± 0.6	0.1 ± 0.3	0.3 ± 0.6	0.1 ± 0.5	0.1 ± 0.3
Trial 2 - duration (secs)	5.3 ± 3.9	4.5 ± 2.9	7.6 ± 8.1	3.9 ± 1.3	4.4 ± 1.9
Females					
Session 1 (PND 60±2) [b]					
Number of animals	16	16	16	16	16
Trials to criterion	7.2 ± 1.9	7.8 ± 3.1	6.9 ± 2.7	7.6 ± 1.8	7.3 ± 2.4
Trial 1- errors	0.7 ± 0.7	0.6 ± 0.5	1.0 ± 1.4	1.0 ± 0.6	0.7 ± 0.9
Trial 1 - duration (secs)	18.1 ± 14.2	13.0 ± 4.9	21.3 ± 18.8	16.3 ± 5.7	14.5 ± 10.7
Trial 2 - errors	0.5 ± 0.6	0.5 ± 0.6	0.7 ± 1.2	0.8 ± 0.9	0.3 ± 0.4
Trial 2 - duration (secs)	14.8 ± 11.7	11.1 ± 8.0	14.4 ± 14.8	14.4 ± 11.2	7.8 ± 4.1
Failed to meet criterion	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (PND 67±2) [b]					
Number of animals	16	15	16	16	16
Trials to criterion	6.2 ± 2.1	5.5 ± 1.1	5.8 ± 1.3	5.9 ± 1.4	6.6 ± 3.4
Trial 1 - errors	0.4 ± 0.9	0.3 ± 0.6	0.2 ± 0.4	0.4 ± 0.6	0.1 ± 0.3
Trial 1 - duration (secs)	8.9 ± 9.5	9.5 ± 7.1	7.8 ± 4.5	9.8 ± 5.4	5.9 ± 3.3
Trial 2 - errors	0.1 ± 0.3	0.0 ± 0.0	0.2 ± 0.5	0.1 ± 0.3	0.1 ± 0.3
Trial 2 - duration (secs)	4.6 ± 3.0	5.4 ± 3.6	4.8 ± 4.0	4.4 ± 2.5	4.5 ± 2.3

[a] Data obtained from pp. 209-210, MRID 46590001
[b] Session 1= learning phase; Session 2 = retention phase

5. **Ophthalmology**: No treatment-related lesions were observed. Corneal opacity, persistent pupillary membrane, retinal degeneration and cataracts were observed at low incidence in both control and treated groups.

6. **Postmortem results:**

   a. **Brain weight:** Mean brain weight data are presented in Table 17. No treatment-related differences between treated and control groups were observed. It should be noted,

however, that the strain of rat used in this study was Wistar Hannover. When fenamidone was tested in Sprague-Dawley rats in a 2-generation reproduction toxicity study (MRID 45400014), which was also used for dose selection for the present study, decreases in absolute brain weights were observed in F1 female adults and F2 female pups.

TABLE 17. Mean (±SD) Brain Weight Data in Offspring [a]

Parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
Day 21 - fixed brain weight (g)	1.38 ± 0.06	1.38 ± 0.05	1.41 ± 0.04	1.43 ± 0.06	1.35 ± 0.06
Day 75 - fixed brain weight (g)	1.82 ± 0.07	1.81 ± 0.11	1.81 ± 0.08	1.75 ± 0.12	1.77 ± 0.10
Day 75 - non-perfused brain weight (g)	1.91 ± 0.08	1.82 ± 0.15	1.83 ± 0.13	1.90 ± 0.13	1.88 ± 0.09
Females					
Day 21 - fixed brain weight (g)	1.36 ± 0.08	1.37 ± 0.10	1.37 ± 0.02	1.36 ± 0.07	1.33 ± 0.05
Day 75 -fixed brain weight (g)	1.70 ± 0.06	1.72 ± 0.09	1.71 ± 0.08	1.68 ± 0.07	1.64 ± 0.04
Day 75 - non-perfused brain weight (g)	1.70 ± 0.13	1.75 ± 0.07	1.76 ± 0.07	1.75 ± 0.09	1.71 ± 0.10

[a] Data obtained from pages 961-962, 964-965, 967-968, MRID 46590001.
N=9-10

b. **Macroscopic examination:** At the PND 21 necropsy, one female in the 4700 ppm group had a moderate pitted zone in the left cerebrum. There were no changes on microscopic examination of this animal and the finding was not reported in other animals at terminal necropsy.

c. **Neuropathology:**

1) **Microscopic examination:** No treatment-related findings were observed either at PND 21 (brain) or at terminal necropsy (brain and spinal cord, both eyes, selected peripheral nerves, gasserian ganglion, gastrocnemius muscle).

2) **Brain Morphometry:** Data are presented in Table 18 for PND 21 and Table 19 for PND 75(±5). On PND 21, there was a significant increase in the caudate putamen measurement in treated males. At the terminal necropsy, a significant decrease in the hippocampal measurement in treated females and an increase in the frontal cortex and cerebellum in treated males were observed. At this time it is not possible to ascertain whether the findings at the high dose are treatment-related, since the historical control data are not adequately described. The registrant is requested to perform morphometric measurements on PND 21 and 75 animals treated with 60, 250, or 1000 ppm fenamidone.

TABLE 18: Mean (±SD) morphometric measurements (mm) in perfused F_1 rats on PND 21[a]

Parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
CrebLn	13.17 ± 0.25	13.20 ± 0.30	13.27 ± 0.31	13.23 ± 0.23	13.07 ± 0.20
CrbellLn	6.61 ± 0.46	6.84 ± 0.37	6.94 ± 0.29	6.79 ± 0.41	6.73 ± 0.36
Frontal Cortex	1.656 ± 0.010	–	–	–	1.725 ± 0.010
Parietal Cortex	1.843 ± 0.002	–	–	–	1.842 ± 0.006
Caudate Putamen	2.959 ± 0.013	–	–	–	3.092 ± 0.017 *
Hippocampus	1.579 ± 0.008	–	–	–	1.559 ± 0.015
Cerebellum	4.081 ± 0.138	–	–	–	4.137 ± 0.026
Females					
CrebLn	13.13 ± 0.13	13.05 ± 0.32	13.09 ± 0.41	13.23 ± 0.43	12.98 ± 0.41
CrbellLn	6.79 ± 0.27	6.85 ± 0.29	6.55 ± 0.28	6.63 ± 0.35	6.57 ± 0.28
Frontal Cortex	1.710 ± 0.006	–	–	–	1.676 ± 0.015
Parietal Cortex	1.846 ± 0.008	–	–	–	1.821± 0.002
Caudate Putamen	2.981 ± 0.027	–	–	–	2.960 ± 0.010
Hippocampus	1.575 ± 0.006	–	–	–	1.538 ± 0.008
Cerebellum	4.123 ± 0.065	–	–	–	4.153 ± 0.165

[a] Data were obtained from pages 961-962 and 970-971, MRID 46590001.
N=10
– = Not examined
CrebLn = Ant/Post Cerebrum Length
CrbellLn = Ant/Post Cerebellum Length
* Significantly different from control, $p<0.05$.

Table 19: Mean (±SD) morphometric measurements (mm) in perfused F_1 rats on PND 75 (±5)[a]

Parameter	Dietary concentration (ppm)				
	0	60	250	1000	4700
Males					
CrebLn	14.38±0.21	14.50±0.28	14.60±0.20	14.48±0.23	14.42±0.15
CrbellLn	7.46±0.20	7.51±0.26	7.42±0.26	7.33±0.26	7.47±0.18
Frontal Cortex	1.576±0.009	–	–	–	1.706±0.013 *
Parietal Cortex	1.827±0.010	–	–	–	1.749±0.118
Caudate Putamen	3.186±0.026	–	–	–	3.280±0.016
Hippocampus	1.787±0.005	–	–	–	1.752±0.009
Cerebellum	4.217±0.068	–	–	–	4.421±0.013 *
Females					
CrebLn	14.16±0.46	14.30±0.40	14.18±0.30	14.35±0.27	14.10±0.27
CrbellLn	7.39±0.24	7.66±0.27	7.53±0.41	7.59±0.23	7.31±0.30
Frontal Cortex	1.701±0.004	–	–	–	1.660±0.009
Parietal Cortex	1.779±0.010	–	–	–	1.797±0.004
Caudate Putamen	3.213±0.019	–	–	–	3.270±0.024
Hippocampus	1.759±0.010	–	–	–	1.677±0.004 *
Cerebellum	4.283±0.023	–	–	–	4.377±0.029

[a] Data were obtained from pages 964-965 and 973-974, MRID 46590001.
N=10
– = Not examined
CrbellLn = Ant/Post Cerebellum Length
CrebLn = Ant/Post Cerebrum Length
* Significantly different from control, $p<0.05$.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the maternal LOAEL was 4700 ppm based on statistically significantly decreased weight gain during gestation (8%) and decreased body weight (maximum 5%) during lactation. The maternal NOAEL was 1000 ppm. The offspring LOAEL was 4700 ppm based on significantly decreased body weight on PND 4 through 21 (9-10%), decreased body weight gain from PND 0 through 21 (9%) and significantly decreased body weight (3-6%) during the first three weeks post-treatment. The offspring NOAEL was 1000 ppm.

B. **REVIEWER COMMENTS:** Administration of technical grade Fenamidone in the diet at concentrations of up to 4700 ppm resulted in no maternal deaths during the study. No treatment-related clinical signs were observed during the cage-side observations or FOB testing. Mean body weight during gestation was unaffected by treatment, but mean body weight gain was significantly decreased at 4700 ppm with the most pronounced effect during

GDs 6-13. Food consumption during GDs 6-13 was significantly decreased at 1000 ppm. This decrease in food consumption is considered due to lack of palatability and not a toxic effect of the test article especially since a corresponding effect on body weight was not observed. The significant increase in food consumption during this period in females at 4700 ppm was attributed to food spillage. Actual food consumption in the 4700 ppm could have been reduced since weight gain was markedly affected after introduction of the treated diet and the limit of palatability is reported to be 5000 ppm. Mean body weight during lactation was slightly decreased in females at 4700 ppm, but food consumption was unaffected by treatment. No treatment-related effects on reproductive performance were observed.

Litter viability and clinical signs were unaffected by treatment. No treatment-related effect on mean body weight of offspring was observed at birth; however, body weight was significantly decreased in males and females at 4700 ppm during PNDs 4-21. Body weight gain during the pre-weaning period was also significantly decreased in male and female offspring at 4700 ppm. Post-weaning (PNDs 28-70) body weight was significantly decreased in males and females at 4700 ppm during the first three weeks but body weight gain was not affected by treatment. Attainment of vaginal opening in females and preputial separation in males and pupil constriction response in both sexes was not affected by treatment. No treatment-related effects were observed on FOB, motor/locomotor activity, auditory startle habituation or learning and memory testing (passive avoidance and watermaze performance). Significant differences from control values were sporadic and not dose-related. No treatment-related effects were reported for ophthalmologic examination. At necropsy, brain weight and macroscopic/ microscopic examination of the nervous system were unaffected by treatment. At the PND 21 necropsy, one female in the 4700 ppm group had a moderate pitted zone in the left cerebrum. This finding was not considered treatment-related since there were no changes on microscopic examination of this animal and the finding was not reported in any animals at terminal necropsy. On PND 21, there was a significant increase in the caudate putamen measurement in treated males. At the terminal necropsy, a decrease in the hippocampal measurement in treated females and an increase in the frontal cortex and cerebellum in treated males were observed. None of these findings is considered treatment-related since there was no consistency in the findings, there were no microscopic changes in the regions and the measurements in treated animals were within the respective historical control ranges.

The maternal systemic and neurotoxicity LOAEL for Fenamidone was not determined. The maternal NOAEL is 4700 ppm (429 mg/kg/day).

The offspring systemic and neurotoxicity LOAEL for Fenamidone in rats is 4700 ppm (429 mg/kg/day) based on decreased body weight and body weight gain during pre-weaning and decreased body weight during post-weaning. The offspring NOAEL is 1000 ppm (92.3 mg/kg/day).

This developmental neurotoxicity study is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426. Previously, the Health Effects Division suggested that a preliminary dose-range finding study be conducted if the Crl:CD(SD)BR strain of rat were

not utilized in the required DNT study, since the 2-generation reproduction study (MRID 45400014) was conducted with the Crl:CD(SD)BR strain of rat (Memo, PV Shah, 12-Jan-2004, TXR# 0052296). However, the DNT study was conducted with the Wistar Hannover strain of rat. It is therefore not possible to assess whether the decreases in absolute brain weight observed in the F1 and F2 generations in the 2-generation reproduction study using the Crl:CD(SD)BR strain of rat would also be observed in the required DNT paradigm, using the Crl:CD(SD)BR strain of rat.

C. STUDY DEFICIENCIES:.

1. References were made to positive control studies for neurobehavioral testing (motor activity, acoustic startle habituation, passive avoidance and water maze), but no actual data were presented.

2 The studies to which the historical control data for brain morphometric measurements refer were not adequately described. It is unknown whether the study numbers and data were contemporaneous with and gathered from the same species and strain used in the present study. Therefore, it is not possible to ascertain whether the findings at the high dose are treatment-related. The registrant is requested to perform morphometric measurements on PND 21 and 75 animals treated with 60, 250, or 1000 ppm fenamidone.

3. Homogeneity and stability data of the test article in the diet were not reported.

4. Clinical observations were not recorded for all dams during lactation (n=≥23/30), and body weight and food consumption were not measured for all dams during lactation (n=≥22/30). No explanation was provided for these data omissions.

5. Summary litter data were reported with data from 7 litters having been omitted. Given that the fertility index was approximately 96.7-100% for the 30 dams/dose on study, this omission is inexplicable. It appears from the individual animal data, however, that the size of these 7 litters was in many cases much smaller than the mean. It also appears from the individual animal data that the dams from these 7 litters underwent "elective sacrifice". No explanation was provided.

6. Summary data for trials 1 and 2 only were reported for each session of each test of learning and memory. Summary data for all trials per session should have been included in the study report.

7. The "standardized procedures" for the FOB were not described for either dams or offspring.

8. According to the study methods, body weight was supposed to be measured at attainment of vaginal opening and preputial separation, but no data were reported.

9. The standard error (SE) of the mean is not a measure of data variability. The standard deviation (SD) is the appropriate metric of variability around a mean for normally distributed data and should have been reported for all parameters (see, for example, litter data).