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UNDATED

DATA EVALUATION RECORD

IMIDACLOPRID

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300 MRID 45537501

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 02-65

Primary Reviewer:
Cheryl B. Bast, Ph.D., D.A.B.T

Signature:_____
Date: _____

Secondary Reviewers:
Carol Forsyth, Ph.D., D.A.B.T.

Signature:_____
Date: _____

Robert H. Ross, M.S, Group Leader

Signature:_____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:_____
Date: _____

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EPA Reviewer: K. Raffaele, Ph.D.
Registration Action Branch 3, Health Effects Division (7509C)
EPA Reviewer: W. Sette, Ph.D.
Toxicology Branch, Health Effects Division (7509C)
EPA Work Assignment Manager: G. Dannan, Ph.D.
Registration Action Branch 3, Health Effects Division (7509C)

Signature: _____
Date _____
Signature: _____
Date _____
Signature: _____
Date _____

Template version 11/01

DATA EVALUATION RECORD
TXR#: 00501055

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

PC CODE: 129099

DP BARCODE: D286291
SUBMISSION NO.: S619873

TEST MATERIAL (PURITY): Technical Grade Imidacloprid (98.2-98.4%)

SYNONYMS: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine

CITATION: Sheets, L. P. (2001) A Developmental neurotoxicity screening study with technical grade Imidacloprid in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 110245; September 14, 2001. MRID 45537501. Unpublished

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorne Road, Kansas City, Missouri 64120-0013.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45537501), Imidacloprid (98.2-98.4% a.i., batch # 803-0273) was administered to 30 parent female Wistar rats/group in the diet at concentrations of 0, 100, 250 or 750 ppm from gestation day 0 through postnatal day (PND) 21. The average daily intake of Imidacloprid was 0, 8.0-8.3, 19.4-19.7, and 54.7-58.4 mg/kg/day during gestation and 0, 12.8-19.5, 30.0-45.4, and 80.4-155.0 mg/kg/day during lactation, for the 0, 100, 250, and 750 ppm groups, respectively. A Functional Operational Battery (FOB) was performed on all dams on gestation days 6, 13, and 20 and on 10 dams/dose on lactation days 4, 11, and 21. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring, representing at least 20 litters/dose, were allocated for detailed clinical observations (abbreviated FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of learning and memory, and ophthalmology. Neural tissues were also collected from selected offspring (10/sex/dose representing 20 litters) on PND 11 and at study termination (75 days of age). Pup physical development was assessed by bodyweight, day of surface righting, auditory startle, eye opening, pupillary constriction, vaginal patency in females and balanopreputial separation in males.

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Treatment-related effects for maternal animals were limited to a 9% decrease (NS) in food consumption for dams in the high dose group compared to controls during the third week of gestation and 14% decrease ($p<0.05$) for high-dose animals during week 1 of lactation. There was also a slight decrease in body weight gain (67% of controls) during LD0-7. **The maternal LOAEL for Imidacloprid in rats is 55-58 mg/kg/day in the diet based on decreased food consumption and decreased body weight gain during lactation. The maternal NOAEL is 20 mg/kg/day in the diet.**

Treatment-related effects for offspring were limited to the high dose group. Body weights of high-dose males and females were significantly ($p<0.05$) decreased 9-13% prior to weaning, and from 3-11% after weaning, with recovery: in females to control levels by PND 50; and in males to a 4% difference that persisted to study termination. Body weight gains were also decreased 12-23% during lactation, with recovery by PND 17. Overall motor activity was decreased (not statistically significantly) on PND 17 in high-dose males (38%) and females (31%) and in PND 21 females (37%). High dose females at study termination had a statistically significant ($p <0.03$; t test) decrease in thickness of the caudate/putamen in comparison to controls (3.7504 vs 3.6774 mm (-2%).

The offspring LOAEL for Imidacloprid in rats is 55-58 mg/kg/day in the diet, based on decreased body weight and body weight gain, decreased motor activity, and decreased caudate/putamen width in females. The offspring NOAEL is 20 mg/kg/day.

This study is classified acceptable/ non-guideline and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). The study may be upgradable upon submission of (1) complete analytical data; (2) morphometric measurements for caudate/putamen for females at intermediate dose levels; and (3) additional positive control data, as described below.

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

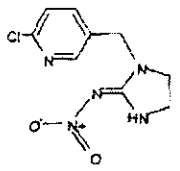
I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Technical grade MKH 3586 Imidacloprid

Description:	Cream colored powder
Lot/Batch #:	803-0273
Purity:	98.2-98.4 % a.i.
Compound Stability:	Confirmed for 21 months
CAS # of TGAI:	138261-41-3

Structure:



2. **Vehicle and/or positive control:** corn oil in the diet

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

Species:	Rat
Strain:	Wistar Crl:W(HAN)BR
Age at study initiation:	females: at least 12 wks; males: at least 15 weeks (breeders only)
Wt. at study initiation:	170.8-231.1 g
Source:	Charles River Laboratories
Housing:	Individually or with litter in stainless steel grid (pre-breeding and post-weaning) or plastic cages (during lactation and gestation)
Diet:	Purina Mills Rodent Lab Chow 5001-4, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 19-25°C **Humidity:** 30-70% **Air changes:** At least 12/h **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least 6 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: July 12, 1999; End: October, 1999

2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day 0 through lactation day 21. Pups were weaned on postnatal day 21, after which time maternal animals were killed. The study included 30 parent females/dose level. F1 pups remained on study up to postnatal days 70-80 (study termination).

3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. After successful mating, each pregnant female was placed into an individual plastic nesting cage, where the dam was maintained through gestation and lactation. Females not found sperm positive within the five-day mating period were sacrificed without necropsy.

4. **Animal assignment:** Mated females and offspring were allocated as shown in Table 1 using an animal allocation program written in SAS. Following delivery, litters for continuation on study were selected as follows (study report, p. 28):

   Litter size (the number of pups delivered) and the "status" of pups at birth were recorded for each litter. If a dam delivered fewer than three pups per sex or if the litter size became

fewer than seven by postnatal day 4, the dam and litter were sacrificed without necropsy examination....If there were more than 23 acceptable litters for any dietary litter, the surplus litters may have been sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females.

For offspring, four sets of animals (designated sets A-D) were utilized for assessment at each age. Randomly-selected pups (10/sex/dose) were designated as Set D and were perfused with fixative and brains were collected for histopathological examination and morphometric analysis.

One pup/sex/litter/group (total of 16 pups/sex/group from 20 litters) was allocated on postnatal day 4 to one of the following: motor activity; acoustic startle habituation; or passive avoidance, water maze, and detailed observational battery. At approximately 50-60 days of age, a minimum of 10 offspring/sex/dose level from sets A, B, and C were given an ophthalmoscopic examination. On day 70-80, these animals were sacrificed by perfusion and neural and muscle tissues collected for microscopic examination.

TABLE 1. Study design

Experimental parameter		Dose (ppm in diet)			
		0	100	250	750
Maternal animals–Main study					
		No. of maternal animals assigned			
No. of maternal animals assigned		30	30	30	30
FOB (GD 6, 13, 20; LD 4, 11, 21)		10	10	10	10
Offspring– Main study					
Set A	Motor activity (PND 13, 17, 21, 58-62)	16/sex	16/sex	16/sex	16/sex
Set B	Acoustic startle habituation (PND 22, 36-40, 58-62)	16/sex	16/sex	16/sex	16/sex
Set C	Passive Avoidance (PND 22, 29)	16/sex	16/sex	16/sex	16/sex
	Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	16/sex	16/sex	16/sex	16/sex
	Water maze (PND 58-62, 7 days after first test. These were the same animals assigned to the passive avoidance test)	16/sex	16/sex	16/sex	16/sex
Sets A-C	Ophthalmologic evaluation (PND 50-60), Gross Necropsy, Perfusion Histopathology, Fixed Brain Weight, Morphometry (PND 70-80)	10/sex	10/sex	10/sex	10/sex
	Fresh Brain Weight (PND 70-80)	10/sex	10/sex	10/sex	10/sex
Set D	Gross necropsy, Histopathology, and Brain Morphometry (PND 11)	10/sex	10/sex	10/sex	10/sex

5. **Dose selection rationale:** Dose levels were chosen based on the results from a two-generation reproduction study in Wistar rats (Report 100647, MRID 42256340, 1990). In that study, Imidacloprid was administered in the diet at levels of 0, 100, 250, and 700 ppm. Effects at 700 ppm included decreased food consumption and body weight gain in parental and F1-generation males and females. There were no other compound-related clinical signs

or effects. Based on these results, the doses selected for the developmental neurotoxicity study were 0, 100, 250, and 750 ppm. The 100 ppm dose was selected to produce no signs of toxicity, and the 250 ppm level was selected as an intermediate dose to assist in establishing compound-related effects and a NOAEL. The 750 ppm dose was selected to produce evidence of toxicity and approximate the MTD.

6. **Dosage administration:** Imidacloprid was administered to parent female Wistar rats (30/dose) in the diet at levels of 0, 100, 250 or 750 ppm from gestation day 0 through postnatal day 21. The average daily intake of Imidacloprid, based on stated analytical values, was 0, 8.0-8.3, 19.4-19.7, and 54.7-54.8 mg/kg/day during gestation and 0, 12.8-19.5, 30.0-45.4, and 80.4-155.0 mg/kg/day during lactation, for the 0, 100, 250, and 750 ppm groups, respectively. The mean analytically-determined concentrations, as stated in the text of the report, were 0. 95.5, 227, and 691 ppm, respectively, for the 0, 100, 250, and 750 ppm groups.

7. **Dosage preparation and analysis:** Detailed descriptions of feed preparations and test diet analysis were not provided; however, information from the study report is as follows. Corn oil was used as the vehicle for the test article at 1% by weight of the diet, and acetone served as a solvent in the diet preparation process and was allowed to evaporate. It was stated that dietary formulations were prepared weekly (stored in the freezer until use), and that concentrations of the test substance in the diet were measured by liquid chromatography three times during the in-life phase of the study, however analytical data were not included in the report (mean concentrations were stated in the text, as noted above). Homogeneity and stability data from a previous study, cited as MRID 43286401 (utilizing concentrations of 50 to 4000 ppm) were cited.

Results:

Homogeneity analysis: was not performed for this study; however the study report states, "At nominal concentrations of 50 ppm and 4000 ppm, Imidacloprid is homogeneous and stable for at least 14 days at room temperature and 28 days at freezer storage conditions."

Stability analysis: was not performed for this study; however the study report states, "At nominal concentrations of 50 ppm and 4000 ppm, Imidacloprid is homogeneous and stable for at least 14 days at room temperature and 28 days at freezer storage conditions."

Concentration analysis: The report stated that the mean analytical concentrations of test diets were 0, 95.5, 227, and 691 ppm, respectively, for the 0, 100, 250, and 750 ppm diets.

The analytical data indicated the concentration of Imidacloprid in the diets was adequate. The use of stability and homogeneity data from a previous study should be confirmed.

C. OBSERVATIONS

1. **In-life observations:**

a. **Maternal animals:** Once daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals.

Ten dams per group were observed (by observers blind to the treatment group) in the home cage, during handling, and outside the home cage in an open field during the gestation dosing period (days 6, 13 and 20) and during the lactation dosing period (days 4, 11 and 21). Observations were conducted using standardized procedures and defined severity scores. The following functional observations were recorded.

	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.
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 data were recorded weekly for gestation days 0-6, 6-13, 13-20, and lactation days 0-7, 7-14, and 14-21.

From gestation day 20, dams were checked daily for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Numbers of live and dead offspring were recorded during parturition.

b. Offspring:

1. **Litter observations:** Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity.

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

2. **Developmental landmarks:** One male and one female from each litter were examined daily for the following landmarks, beginning on the following days: PND 4- surface righting; PND 10- auditory startle response; PND 11- eye opening; PND 21 pupillary constriction; PND 29- vaginal patency in females; and PND 38- balanopreputial separation in males. The age of onset was recorded.

3. **Detailed observations:** Offspring were examined for clinical signs once daily during the preweaning period and once weekly after weaning by observers aware of assignment to the treatment groups. Individual offspring body weight data were recorded on postnatal days 0, 4, 11, 17, and 21 and once weekly thereafter. Individual food consumption was measured weekly from the week of postnatal day 28.

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

4. **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45±1, and 60±2, a total of 16 offspring/sex/group (one male or one female per litter, representing at least 20 litters) was examined outside the home cage in an FOB assessment by observers blind to the treatment groups. On postnatal days 4 and 11, the animals were not evaluated in the open field, unless deemed necessary by the observer. Otherwise, methods were similar to the procedures used for the dams.

FUNCTIONAL OBSERVATIONS- Offspring	
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.
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.

5. **Motor activity testing:** Motor activity was evaluated in 16 rats/sex/dose on days 13, 17, 21, and 60. Animals were placed individually in figure-eight mazes and were continuously monitored over a 1-hour period. An automated activity monitoring system collected data over successive 10-minute intervals by recording infra-red light source break frequency within the maze. Motor activity was measured as the number of beam interruptions that occurred during the test session, and locomotor activity was measured by eliminating consecutive counts for a given beam. Therefore, only one interruption of a given beam was counted for locomotor activity until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrease in activity over consecutive test-session intervals. Broad spectrum background noise (74 ± 2dB(A)) was provided throughout testing to minimize acoustical variation. Uniformity of light intensity (100±70 Lux) over each maze was verified daily.

6. **Auditory startle habituation:** Auditory startle reflex habituation was performed on 16 offspring/sex/dose on postnatal days 22, 38±2, and 60±2, using an automated system (Coulbourne Instruments, Allentown, PA).

   Animals were acclimated for 5 minutes to background noise and were then presented with the startle stimulus at 10-second intervals for 50 trials. The startle stimulus consisted of 50-millisecond bursts of white noise at approximately 120 dB. Peak response amplitude (g force exerted on the platform) and latency (msec) measurements were recorded for each animal's individual response curve. Response amplitude was defined as the maximum value of the average curve minus the baseline (correcting for body weight). Latency to peak was defined as the time, in msec, following onset of the stimulus when the peak response amplitude occurred.

7. **Learning and memory testing:**

   PASSIVE AVOIDANCE CONDITIONING: On postnatal days 24 and 31, learning and short- and long-term retention were assessed in a passive avoidance test using 16 offspring/sex/dose. Testing was done in individual isolation cubicles each with a single shuttle cage (Coulbourne Instruments, Allentown, PA). Each cubicle was insulated to attenuate sound and had a fan for ventilation. Each 7 x 7 inch shuttle cage was separated into two equal-sized compartments by a centrally-located sliding door. The two compartments were identical except that the walls in one compartment were lined with black film (dark side) and the walls in the other compartment were not lined and this compartment was illuminated with a high-intensity lamp. The lamp was switched on at the beginning of each trial and remained on until the rat crossed into the dark compartment or the trial ended. The cage floor was constructed of a stainless steel grid and the movement of the rat from the light to dark side was detected by a photocell. Rats were placed individually into the shuttle cage facing toward the light. After 20 seconds, the light was switched on and the door separating the compartments was opened. When the rat crossed into the dark side, the door closed, a brief, mild shock (0.5 sec, 0.5mA) was delivered, and the light was switched off. If the rat failed to cross to the dark side within 180 seconds, it was returned to the holding cage and assigned a latency time of 180 sec. The procedure was repeated (Inter-trial interval was not provided) until the rat either remained in the bright side for 180 seconds for two consecutive trials or until 15 trials had elapsed (whichever occurred first). Rats that failed to reach

criterion performance within 15 trials or failed to cross during the first two acquisition trials were excluded from the retention phase of the experiment (conducted 1 week later, on day 31; procedure was the same as for the learning phase).

WATER MAZE: Learning and memory testing was performed in 16 offspring/sex/dose on postnatal days 60 and again seven days later (retention phase) using an M-water maze. Only rats that demonstrated acquisition on the first test occasion were tested for retention seven days later. The water maze was made of opaque Plexiglas with 5-inch wide corridors. The walls were 16-inches high with approximately 7.5 inches of water. The maze was filled with water at 22±1°C. For each test trial, the rat was placed at the base of the M-maze stem, between the two lateral arms. On the learning trial (first trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and was then removed from the maze. The initial arm chosen on the learning trial was designated the incorrect goal during the subsequent trials (15 maximum). Rats failing to make a correct goal choice within 60-seconds in any given trial were led to the correct goal with the exit ramp and then removed from the water. The inter trial interval was approximately 15 seconds. Each rat was required to reach a criterion of 5 consecutive errorless trials to stop the test session. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as well as the number of errors (incorrect turns, not otherwise defined) during each trial.

8. **Ophthalmology:** At 50-60 days of age, indirect ophthalmoscopy was performed on 10 offspring/sex/dose (that had been selected for perfusion) following dilation with a mydriatic agent.

9. **Postmortem observations:**

   a. **Maternal animals:** Maternal animals were sacrificed by carbon dioxide inhalation, following weaning on postnatal day 21. Adult females were not routinely subjected to a gross necropsy. Maternal animals found moribund were sacrificed and necropsied. Those found moribund or dead were subjected to a macroscopic necropsy, with possible collection of tissues at the discretion of the study director.

   b. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 11 or 70-80. These animals were subjected to postmortem examinations as described below. For both PND 11 and PND 75 animals, a step down procedure and a subjective diagnosis procedure as described in the adult neurotoxicity guidelines was followed. First, high dose and control animals were examined. If no treatment related effects were seen, no further examination occurred. If effects were seen in a region, sections from all dose groups were coded, examined blindly, with the frequency and severity of each lesion scored. The code was then broken, and the data examined for dose response relationships.

   PND 11: At postnatal day 11, 10 pups/sex/dose from Set D were selected for gross necropsy and brain weight measurements. Animals were sacrificed by intraperitoneal injection of Fatal Plus and then underwent a gross necropsy examination. The calvaria

were sliced at the top of the skull to expose the brain and the entire head was immersed in 10% buffered formalin for 24 hours. The brain with olfactory bulbs was removed and weighed. Anterior to posterior cerebrum and cerebellum length were measured by a technician not blind to treatment using a Vernier caliper. The remaining pups assigned to Set D were sacrificed without necropsy.

After the gross brain measurements were recorded, brains from control and high-dose rats were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin, luxol fast blue/cresyl violet and Sevier-Munger stains. Eight coronal sections from control and high-dose animals were examined microscopically.

The following 7 brain morphometric measurements were made for control and high-dose animals:

1. Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm);
2. Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm);
3. Caudate putamen and underlying globus pallidus horizontal width (coronal section taken at the level of the optic chiasm);
4. Corpus callosum (thickness at the midline);
5. Hippocampal gyrus (greatest dorsal-ventral thickness from the ventral tail of the dentate gyrus to the overlying subcortical white matter);
6. Cerebellum height (roof of the fourth ventricle to the dorsal surface);
7. External germinal layer (multiple areas were measured over the dorsum of the cerebellum and the mean value taken as one measurement).

On postnatal day 70-80 (Sets A-C), 10 animals/sex/group were euthanized by carbon dioxide asphyxiation, underwent a gross necropsy and the brains were removed and weighed (fresh weight) and discarded. Another 10 rats/sex/dose were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg) and perfused via the left ventricle with a sodium nitrite flush followed by fixation with 10% buffered formalin. The brain, spinal cord, both eyes with optic nerves, peripheral nerves, gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected, weighed (brain only), and post-fixed with 10% buffered formalin. Anterior to posterior cerebrum and cerebellum length were measured by an individual not blind to treatment using a Vernier caliper.

The following central and peripheral nervous tissues from perfused animals were dissected and preserved in paraffin (CNS tissues) or Glycol methacrylate (PNS tissues): eight coronal sections of the brain, cervical, thoracic, and lumbar sections of the spinal cord, the cauda equina, eyes, optic nerves, gastrocnemius muscle, dorsal root ganglia and fibers, and gasserian ganglion. Tissues from all dose groups were embedded; however, only control and high-dose tissues were examined unless effects warranted examination of low- and mid-dose samples. Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin. Glycol methacrylate-embedded tissues were sectioned at 2-3 μm and stained with a modified Lee's stain.

Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was conducted as follows:

1. Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm);
2. Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)
3. Caudate putamen and underlying globus pallidus horizontal width (coronal section taken at the level of the optic chiasm)
4. Corpus callosum (thickness at the midline)
5. Hippocampal gyrus (greatest dorsal-ventral thickness from the ventral tail of the dentate gyrus to the overlying subcortical white matter)
6. Cerebellum height (roof of the fourth ventricle to the dorsal surface)

C. DATA ANALYSIS:

1. **Statistical analyses:** Continuous data were initially analyzed for equality of variance using Bartlett's test. Group means with equal variances were further analyzed with ANOVA, followed by Dunnett's test if significance was identified with the ANOVA. Group means with unequal variances were analyzed by Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons. The level of significance was set at $p \leq 0.05$, except for Bartlett's test which was set at $p \leq 0.001$.

   Motor and locomotor activity were analyzed with ANOVA, followed by Dunnett's test if significance was attained with ANOVA; repeated-measures ANOVA was used to analyze interval data. Acoustic startle peak amplitude data were analyzed by ANOVA followed by Dunnett's test if significance was observed with the ANOVA. The response amplitude data for each block of 10 trials were subjected to a Repeated-Measures ANOVA, using the test block as the repeated measure. Passive avoidance latency data were analyzed non-parametrically: Wilcoxon Test was used to analzye latency data, Kruskal Wallis and Wilcoxon tests were used to analyze trials-to-criterion data during acquisition, Fisher's Exact test was used to analyze trials-to-criterion data for retention. Water maze data were analyzed by a univariate ANOVA followed by Dunnett's test. Micropathology frequency data were analyzed by Chi-Square followed by Fisher's Exact Test if significance was identified with the Chi-Square.

2. **Indices:**

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

   Gestation index = (Number of females delivered/Number pregnant) x 100

   Mating index = (Number of inseminated females/Number of females co-housed with males) × 100

   Fertility index = (Number of pregnant females/Number of inseminated females) × 100

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

Live birth index = (Number of live pups born per litter/Total number of pups per litter) × 100

Viability index = (Number of live offspring at PND 4 per litter/Number of live offspring born per litter) × 100

Lactation index = (Number of live offspring on Day 21 per litter/Number of live offspring on PND 4 after culling per litter) × 100

3. **Positive control data:** Positive control data have been submitted and are currently under review.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** There were no maternal deaths before scheduled termination, and there were no treatment-related clinical signs or functional observations observed during gestation or lactation. There was a slight, dose-related, increase in rearing on LD11 (number of rears was 2.0, 2.8, 3.2, and 3.8 for control, low, mid, and high dose, respectively, n.s.). There was a statistically significant increase in urination at the mid-dose on LD21 (number of of pools was 0.5, 0.7, 1.3, and 0.6 for control, low, mid, and high dose, respectively, $p<0.05$); this finding was considered sporadic, and not treatment-related.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 2. There were no treatment-related effects on body weight or body weight gain during gestation. There were also no statistically significant effects on body weight during lactation; body weight gain during lactation was not statistically evaluated. We note, however, that body weight gain for controls from LD0 to LD7 was 21.2 g, compared to a gain of 14.1 g at the high dose (67% of control levels). This difference is consistent with the decreased food consumption by high dose dams during this time period (see below).

Food consumption was decreased 9% (NS) for high-dose dams compared to controls during the third week of gestation and 14% ($p<0.05$) for high-dose animals during week 1 of lactation. No other food consumption effects were noted.

TABLE 2. Selected Mean (±SE) maternal body weight and food consumption[a]				
Observations/study interval	Dose (ppm)			
	0	100	250	750
Gestation (n= 28-30)				
Body wt. Gestation day 0 (g)	204.1±2.48	210.4±2.63	206.2±2.53	206.4±2.80
Body wt. Gestation day 6 (g)	223.4±2.54	230.1±3.08	226.1±2.60	221.5±3.02
Body wt. Gestation day 13 (g)	249.3±2.99	255.5±2.98	249.4±2.76	244.7±2.97
Body wt. Gestation day 20 (g)	308.7±3.97	319.8±3.73	313.7±4.32	307.6±4.58
Wt. gain gestation days 0-20 (g)	104.5±2.38	109.4±2.04	107.4±2.62	101.3±2.67
Food consumption gestation days 0-6 (g/kg/day)	100.5±7.52	83.4±2.39	86.7±2.56	84.5±5.59
Food consumption gestation days 6-13 (g/kg/day)	87.2±1.27	86.8±1.26	85.5±1.42	82.7±1.64
Food consumption gestation days 13-20 (g/kg/day)	87.1±1.63	87.1±0.95	85.3±0.75	79.2±1.31
Lactation (n=28-30 [LD0], 21-25 [LD4-21])				
Body wt. lactation day 0 (g)	238.6±2.51	246.4±2.85	237.2±2.86	233.0±3.27
Body wt. lactation day 4 (g)	254.6±3.49	261.5±4.02	246.9±3.68	241.7±4.92
Body wt. lactation day 7 (g)	259.8±4.02	266.6±3.94	255.6±3.44	247.1±3.93
Body wt. lactation day 11 (g)	269.8±6.01	276.5±3.27	268.0±3.84	263.6±3.34
Body wt. lactation day 14 (g)	271.1±3.50	279.2±4.46	268.8±3.98	262.8±4.17
Body wt. lactation day 21(g)	266.9±4.90	277.0±3.35	266.0±3.83	268.1±3.17
Food consumption lactation days 0-7 (g/kg/day)	135.4±3.72	134.1±3.99	131.9±4.11	116.3**±3.23
Food consumption lactation days 7-14 (g/kg/day)	181.1±4.06	180.5±1.97	186.4±3.54	186.3±6.19
Food consumption lactation days 14-21 (g/kg/day)	202.9±3.88	204.0±4.50	200.1±3.38	224.4±8.56

[a]Data obtained from Tables 3 & 4 pages 60-63 and Tables 6 & 7 pages 66-69, MRID 45537501. **p<0.01.

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. No treatment-related effects were noted.

TABLE 3. Reproductive performance [a]				
Observation	Dose (ppm)			
	0	100	250	750
Number Mated	30	30	30	30
Number Delivered	28	30	30	28
Mating Index (%)	100	100	100	100
Fertility Index (%)	93.3	100	100	93.3
Gestation index (%)	100	100	100	100

[a]Data obtained from Table 1, pages 56-57, and from individual data, pp. 238-241, MRID 45537501.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) during lactation for pups from litters selected for continuation on study are summarized in Table 4. There was no treatment-related effect on the number of litters, live litter size, number of stillborn pups, live

birth index, or viability index. Data for all litters were evaluated, and results for unselected litters were similar to those from selected litters.

TABLE 4. Litter size and viability [a]

Observation	Dose (ppm)			
	0	100	250	750
Number of Litters	21	25	23	23
Total number born	223	282	254	265
Number born live	223	281	254	264
Number born dead	0	1	0	1
Mean No. of viable pups:				
Day 0	11	11	11	11
Day 4 [b]	11	11	11	11
Day 4 [c]	8	8	8	8
Day 21[d]	6	6	6	6
Live birth index (%)	100	99.6	100	99.7
Viability index	100	98.9	98.9	98.6
Lactation index[d]	75.5	75.5	74.8	73.9

[a]Data obtained from Table 9, pages 73-75, MRID 45537501; includes only litters selected for continuation throughout study.
[b]Before standardization (culling).
[c]After standardization (culling).
[d]Two pups/litter were sacrificed on day 11 for histopathological evaluation.

2. **Body weight:** Pre-weaning pup body weight data are presented in Table 5. Body weights were comparable at birth across all dose groups; however, by PND 4, body weight was decreased 9-12% ($p<0.05$ or $P<0.01$) for high-dose male and female offspring compared to controls; this decrease averaged 12-13% on PND 11, and 11% in both sexes ($p<0.01$) by weaning on PND 21. Body weight gain was also decreased in high-dose offspring during lactation. From birth to PND 4, high-dose animals gained approximately 22% less than controls. Lower body weight gain for high-dose animals persisted through PND 17 and appeared to recover to control levels during the period PND 17-21. No treatment-related effects on body weight were noted in mid or low dose pups.

TABLE 5. Mean (±SE) pre-weaning pup body weights and body weight Gain (g) [a]								
Postnatal Day	Dose (ppm)							
	0	100	250	750	0	100	250	750
	Males				Females			
0	5.9±0.09	5.8±0.09	5.9±0.10	5.8±0.10	5.7±0.09	5.5±0.09	5.6±0.10	5.5±0.10
4 [b]	9.5±0.23	9.4±0.22	9.4±0.24	8.6*±0.25 (9%)[d]	9.3±0.21	9.0±0.21	9.2±0.21	8.2**±0.24 (12%)[d]
4 [c]	9.5±0.23	9.3±0.24	9.5±0.25	8.6*±0.25 (9%)[d]	9.2±0.21	9.0±0.22	9.2±0.23	8.2**±0.24 (11%)[d]
11	22.1±0.54	22.5±0.44	22.1±0.49	19.4**±0.50 (12%)[d]	21.6±0.55	22.0±0.43	21.6±0.50	18.7**±0.49 (13%)[d]
17	36.2±0.73	36.7±0.61	36.0±0.70	31.6**±0.72 (13%)[d]	35.2±0.74	35.7±0.52	35.1±0.63	30.9**±0.68 (12%[d])
21	46.2±1.03	46.4±0.74	45.2±1.00	41.1**±0.98 (11%)[d]	44.8±1.05	45.1±0.60	44.0±0.93	40.0**±0.92 (11%)[d]
Weight gain Days 0-4	3.6±0.18	3.6±0.15	3.6±0.17	2.8*±0.19 (22%)[d]	3.5±0.17	3.5±0.14	3.6±0.15	2.7**±0.18 (23%)[d]
Weight gain Days 4-11	12.6±0.44	13.2±0.28	12.6±0.32	10.7**±0.33 (15%)[d]	12.4±0.47	13.0±0.25	12.4±0.35	10.5**±0.32 (15%)[d]
Weight gain Days 11-17	14.0±0.35	14.2±0.28	14.0±0.36	12.3**±0.39 (12%)[d]	13.6±0.34	13.7±0.23	13.5±0.41	12.2*±0.37 (10%)[d]
Weight gain Days 17-21	10.0±0.51	9.7±0.42	9.1±0.48	9.4±0.43	9.6±0.54	9.4±0.36	8.8±0.54	9.1±0.38

[a] Data obtained from Tables12-13, pages 82-90, MRID 45537501. *p<0.05, **p<0.01.
[b] Before standardization (culling).
[c] After standardization (culling).
[d] (%) decrease compared to controls, calculated by reviewer

Post-weaning offspring body weight data are presented in Table 6. Body weights were decreased in high-dose males and females compared to controls following weaning. For males body weights were approximately 11% less than controls at day 29 (the first time following weaning), with partial recovery to 4% less than controls at study termination. For females, body weights averaged 3-8% less than controls for the first 3 weeks after weaning, followed by recovery the last 4 weeks. While a number of statistically significant increases of 3-4% in body weight were seen in low dose males between PND 50-71, and in low and mid dose females between PND 43-71, these were not considered to be biologically significant because of their small magnitude and lack of dose response at higher doses. No other effects on body weight were noted for low- or mid-dose offspring.

TABLE 6. Mean (±SD) post-weaning pup body weights (g) [a]								
Postnatal Day	Dose (ppm)							
	0	100	250	750	0	100	250	750
	Males				Females			
29	69.5±7.3	70.4±5.7	68.5±7.3	62.0*±6.6 (11%)	69.8±7.0	70.8±4.9	70.7±6.7	64.3*±6.4 (8%)
36	114.2±11.0	116.9±9.6	115.4±10.6	107.0*±9.5 (6%)	108.0±9.6	110.5±7.5	109.8±9.9	101.2*±8.2 (6%)
43	160.9±13.3	165.7±12.6	160.9±14.4	151.5*±12.2 (6%)	133.5±10.8	138.5*±9.1	139.2*±11.4	128.9*±9.0 (3%)
50	202.3±14.6	209.4*±15.5	204.3±16.7	193.9*±15.6 (4%)	150.9±11.7	157.4*±10.6	155.8±14.0	147.6±10.8
57	247.4±16.9	255.9*±18.7	248.5±21.5	236.7*±19.5 (4%)	168.3±12.7	173.0±12.6	172.8±14.6	163.6±12.7
64	282.2±18.7	293.7*±22.1	282.0±22.9	271.3*±21.8 (4%)	181.7±13.4	186.0±12.9	185.9±15.8	177.0±13.2
71	308.1±20.0	320.5*±24.6	310.0±25.4	297.1*±24.2 (4%)	191.4±14.8	198.8*±14.5	195.8±17.1	189.6±14.4

[a] Data obtained from Table 15, pages 93-95, MRID 45537501. *p<0.05. There appears to be an error in Table 15 with regard to PND values. This error has been corrected by the reviewer and is reflected in Table 6 of this DER.
Number in parentheses = % decrease compared to controls, calculated by reviewer

No biologically-significant, treatment-related post-weaning food consumption effects were noted. Males in all treatment groups appeared to show statistically significant increases in food consumption; however, these differences were small (6-7%), not dose dependent, and thus not considered biologically significant. Mid- and high-dose females also showed significantly decreased ($p<0.05$) food consumption (10-15%) on days 38 and 45 only. This was considered an isolated finding.

3. **Developmental landmarks:**

   a. **Sexual maturation:** Preputial separation for high-dose males was not delayed relative to controls. There were no treatment-related effects on the mean age for attainment of vaginal opening for females. The data are presented in Table 7.

TABLE 7. Mean (±SD) age of sexual maturation (days) [a]				
Parameter	Dose (ppm)			
	0	100	250	750
N (M/F)	21/21	23/23	20/20	22/22
Preputial separation (males)	45.2±0.45	45.5±0.28	44.9±0.47	44.8±0.38
Vaginal opening (females)	33.1±0.30	33.1±0.25	33.0±0.21	34.4±0.47

[a] Data obtained from Table 14, pages 91-92, MRID 45537501.

   b. **Surface Righting, eye opening, auditory startle, and pupil constriction:** No treatment-related effects were noted with regard to these developmental landmarks.

4. **Behavioral assessments:**

   a. **Functional observational battery:** There were no treatment-related effects at any dose level on any test day (PND 4, 11, 21, 35, 45, or 60).

b. **Motor and locomotor activity**: Session means for motor activity (total beam breaks) and locomotor activity(different consecutive beam breaks) are shown in Tables 8 and 9, respectively. In high dose rats, on PND17 overall motor activity was decreased 38% in males and 31% in females; on PND 21 overall motor activity was decreased 37% in females. (Similar decreases in locomotor activity was seen: 39% and 37% for high-dose males and females on PND 17 and 26% in high-dose females on PND 21). None of these changes were statistically significant. Coefficients of variation for the motor activity data varied between 44-72% for males, and 31-87% for females, with the greatest variability on PND 13 and the least on PND 60. These effects were considered treatment related because of their magnitude and because they occurred in both sexes.

Statistically significant differences in within session activity levels were seen: for high dose females on PND 17, there was a significant decrease during the first interval in both motor activity (47%) and locomotor activity (48%); and for high dose males on PND 60, there was a significant decrease in the first interval for locomotor activity (20%) . In the day 60 males, due to the isolated nature of the change and its relatively small magnitude this effect is not considered toxicologically significant. But for the high dose PND 17 females, where overall activity was reduced 31% and locomotor activity 37%, the decrease in the first interval helps to characterize the overall decreases.

Habituation of motor activity was seen on all test days, even on PND 13 when activity levels were low.

TABLE 8. Mean (±S.D.) motor activity data (total activity counts for session) [a]

Test Day	Dose (ppm)			
	0	100	250	750
Males				
PND 13	184±133 [72%]	125±70 (-32)	151±122 (-18)	162±146 (-12)
PND 17	318±200 [63%]	342±220 (+8)	280±153 (-12)	196±117 (-38)
PND 21	333±149 [45%]	393±184 (+18)	374±213 (+12)	343±202 (+3)
PND 60	586±257 [44%]	628±123 (+7)	556±180 (-5)	566±150 (-3)
Females				
PND 13	126±109 [87%]	141±107 (+12)	153±67 (+21)	130±86 (+3)
PND 17	290±150 [52%]	215±108 (-26)	292±204 (+1)	201± 127 (-31)
PND 21	416±264 [64%]	375±233 (-10)	346±157 (-17)	262±117 (-37)
PND 60	724±224 [31%]	747±388 (+3)	674±232 (-7)	670±239 (-7)

[a] Data obtained from Table 19, pages 197-199, MRID 45537501
N = 13-16/sex/dose.
Number in parentheses = % greater (+) or less (-) compared to control, from p. 46, MRID 45537501.
Numbers in brackets [ ] are coefficients of variation.

TABLE 9. Mean (±S.D.) locomotor activity data (total activity counts for session) [a]				
Test Day	Dose (ppm)			
	0	100	250	750
Males				
PND 13	21±29	10±9 (-52%)	20±35	13±31 (-38%)
PND 17	71±56	71±46	67±46	43±34 (-39%)
PND 21	86±40	96±50	83±31	78±40
PND 60	359±156	375±109	327±114	342±109
Females				
PND 13	16±18	20±34	20±29	13±16
PND 17	68±39	59±43	65±45	43±25 (-37%)
PND 21	89±40	81±36	84±36	66±22 (-26%)
PND 60	407±144	386±205	397±141	375±141

[a] Data obtained from Table 20, pages 200-202, MRID 45537501.
N = 13-16/sex/dose.
Number in parentheses = % greater (+) or less (-) compared to control

c. **Auditory startle reflex :** There was no treatment-related effect noted for peak latency at any dose on any test day; values were similar across dose, time point, and sex. There was a statistically significant ($p<0.05$) increase in peak amplitude for low-dose females on PND 60 (significantly increased mean value and for blocks 2-5; overall mean was 184% of control value). Peak amplitude was also increased for mid-dose females, but the increase was significant only for block 5, and was slightly smaller than for low dose females (mean peak amplitude 170% of controls). For high dose females, a slight increase over controls remained, smaller than the increase for mid-dose females (mean peak amplitude 126% of controls), and not statistically significant for any block. In the absence of any dose-relationship, and given the similarity of high dose and control values, the significant increase in amplitude for low dose females is considered spurious.

Habituation was evident in control males and females on all test days as a decrease in response amplitude over the test session; the degree of habituation (both in terms of absolute value and as a percent of control values) increased with increasing age. Peak amplitude data are summarized in Table 10 and latency data are summarized in Table 11.

TABLE 10. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]					
	Trial Block	Dose (ppm)			
		0	100	250	750
Males					
PND 22	1	46±18	51±28	46±13	37±12
	2	42±15	48±26	45±15	38±13
	3	37±11	47±27	40±15	37±18
	4	39±14	43±24	43±19	35±18
	5	38±17	40±27	39±13	30±15

TABLE 10. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	100	250	750
	Mean	40±13	46±25	43±13	35±14
PND 38	1	137±84	139±78	161±76	111±40
	2	140±86	140±91	154±72	125±59
	3	113±62	105±71	137±67	113±51
	4	109±53	92±53	111±54	94±47
	5	87±50	83±45	104±47	71±42
	Mean	117±61	112±63	133±59	103±43
PND 60	1	327±178	347±208	420±209 (128)	395±170 (120)
	2	340±209	331±248	400±217	399±196
	3	281±175	260±184	307±216	297±158
	4	241±176	202±138	285±194	263±162
	5	190±113	203±124	263±160	192±109
	Mean	276±154	269±171	335±189 (121)	309±141 (112)
Females					
PND 22	1	50±22	45±15	51±21	43±15
	2	48±17	48±17	46±17	45±22
	3	38±15	41±14	45±17	40±18
	4	40±14	38±8	43±14	36±16
	5	36±16	40±13	40±17	34±15
	Mean	42±15	42±11	45±15	40±16
PND 38	1	92±48	115±58	99±61	72±34
	2	81±38	101±58	100±71	78±42
	3	64±32	92±48	85±52	68±35
	4	64±37	80±36	73±48	58±36
	5	59±33	78±46	69±51	49±24
	Mean	72±33	93±45	85±55	65±31
PND 60	1	151±123	214±114	205±137	149±96
	2	108±108	228*±133	195±123	159±97
	3	78±65	176*±102	155±114	112±91
	4	70±35	140*±88	131±72	89±84
	5	59±32	100*±50	107*±57	75±45
	Mean	93±66	171±85* (184)	158±91 (170)	117±77 (126)

[a]Data obtained from Tables 23-24, pages 221-230, MRID 45537501, values in parenthesis are percent of control value.
*p<0.05, ANOVA
N = 16/sex/dose

TABLE 11. Auditory startle latency to peak data (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	100	250	750
Males					
PND 22	1	37±7	39±10	40±7	40±10
	2	39±9	37±8	37±7	38±10
	3	38±9	37±8	39±8	41±9

TABLE 11. Auditory startle latency to peak data (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	100	250	750
	4	39±8	37±9	36±9	38±8
	5	40±11	37±9	36±7	38±7
	Mean	39±8	38±7	37±6	39±7
PND 38	1	32±3	32±5	32±3	30±4
	2	30±4	30±3	31±3	30±2
	3	31±3	32±4	31±3	30±3
	4	31±4	32±4	32±4	30±2
	5	32±5	33±6	33±4	34±5
	Mean	31±3	32±4	32±2	31±2
PND 60	1	38±4	37±4	36±3	36±3
	2	34±3	34±4	36±3	35±2
	3	35±3	34±4	35±2	34±3
	4	35±3	36±4	35±3	34±3
	5	35±4	35±4	35±3	35±4
	Mean	35±2	35±3	35±2	35±2
		Females			
PND 22	1	36±6	37±7	37±5	37±7
	2	37±9	39±11	36±10	34±5
	3	36±9	38±8	37±9	34±7
	4	35±7	40±8	35±9	35±7
	5	35±7	36±7	37±9	34±6
	Mean	36±7	38±6	36±7	35±5
PND 38	1	35±5	33±4	33±4	36±7
	2	32±5	32±4	32±6	34±7
	3	33±6	32±5	30±4	33±5
	4	34±6	31±4	34±5	34±5
	5	34±5	33±7	36±8	36±6
	Mean	33±4	32±3	33±3	35±4
PND 60	1	39±6	38±4	38±5	40±7
	2	38±6	35±4	36±4	38±7
	3	35±6	37±6	36±6	39±7
	4	40±6	38±6	37±6	37±4
	5	39±6	37±5	38±6	40±5
	Mean	38±5	37±3	37±5	39±5

[a]Data obtained from Tables 23-24, pages 221-230, MRID 45537501.
N = 16/sex/dose

d. Learning and memory testing:

Passive avoidance: In general, performance was similar among treated and control groups for both sexes. There was an apparent increase in trial one latency, for session 1, across all doses in males (130-158% of control levels, not statistically significant), with a smaller increase in females (up to 139% of control levels). For males, variability was also increased for this parameter. Since session 1-trial 1 latency records the time between first placement in the bright chamber and first entry into the dark chamber, increases in

this parameter do not indicate a deficit in learning or memory. Although it is possible that this difference in Session 1/Trial 1 latency is treatment-related, there was no dose response relationship (mid-dose animals were more similar to controls, low dose more similar to high dose, for both sexes); there was also no effect on learning during session one (latency trial 2 and trials to criterion for session one were similar for all treated and control groups) or on retention during session two (trials to criterion, and latency for both trials was similar for all treated and control groups). Therefore, the possible differences in session1/trial 1 latency are not considered adverse. Data are summarized in Table 12.

TABLE 12. Passive avoidance performance at PND 24/31(mean ± S.D.) [a]

Test Day/Parameter		Dose (ppm)			
		0	100	250	750
Males					
Session 1 (Learning)	Trials to criterion	3.5±0.9	3.4±0.6	3.1±0.3	3.6±0.7
	Latency trial 1 (sec)	29.4±18.5	44.9±30.8 (153)	38.3±39.7 (130)	46.5±53.4 (158)
	Latency trial 2 (sec)	157.4±40.4	163.8±32.0	175.5±12.8	161.2±37.8
	Failed to Learn/No. Tested	0/16	0/16	0/16	0/16
Session 2 (Retention)	Trials to criterion	2.3±0.6	2.6±0.9	2.3±0.6	2.6±0.8
	Latency trial 1 (sec)	167.4±28.3	173.8±18.9	177.3±9.1	165.7±34.4
	Latency trial 2 (sec)	169.8±40.6	169.7±24.4	176.6±13.6	164.3±43.2
Females					
Session 1 (Learning)	Trials to criterion	3.6±0.7	3.6±1.0	3.9±1.5	3.7±1.2
	Latency trial 1 (sec)	21.7±16.1	30.0±24.2 (138)	20.1±18.9	30.2±23.0 (139)
	Latency trial 2 (sec)	162.1±36.5	158.0±37.4	151.8±50.6	164.2±33.4
	Failed to Learn/No. Tested	0/16	0/16	0/16	0/16
Session 2 (Retention)	Trials to criterion	2.6±0.7	3.0±1.3	2.3±0.6	2.4±0.7
	Latency trial 1 (sec)	150.5±49.7	136.4±54.4	166.2±35.6	170.4±22.7
	Latency trial 2 (sec)	168.6±32.4	162.5±45.2	175.0±20.0	171.4±24.9

[a] Data obtained from Table 25, pages 231-233, MRID 45537501. Values in parenthesis represent percent of control value. N=16/sex/group.

Water maze: There was a statistically significant increase ($p<0.05$) in the number of errors for low- and high-dose males during the first acquisition trial (0.9 and 1.2 errors, respectively, compared to 0.3 in controls), as well as an increase in trial duration ($p<0.05$) in the high dose group only (27.5 sec as compared to 11.6 for controls). Although Trial 1 errors were not significantly increased in mid-dose males (0.8 errors), the value was similar to that seen in low dose males. For females, trial 1 errors were similar across all groups (0.5-0.7 errors). We note that the number of errors for mid and low dose males was similar to that for control and low dose females, therefore the increase in errors is not considered treatment-related for low dose males. However, the mean value for high dose males is outside the range of other groups, both male and female, and the trial duration is also significantly increased, therefore the effect is considered treatment-related for high dose males only. We also note that the interpretation of this finding is unclear, since the increase was seen for trial one only. No differences in trials to criterion or trial 2 errors or duration, or in any of the retention measures (session 2), were seen for either sex.

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TABLE 13. Water maze performance at PND 60/67

Test Day/Parameter		Dose (ppm)			
		0	100	250	750
Males					
Session 1 (Learning)	Trials to criterion	7.8±2.7	7.9±2.7	8.2±2.9	7.8±3.0
	Trial 1 errors (mean ± SD)	0.3±0.4	0.9*±1.0	0.8±1.1	1.2*±1.2
	Trial 1 duration (sec) (mean ± SD)	11.6±4.5	19.8±13.3	18.6±12.0	27.5*±18.7
	Trial 2 errors (mean ± SD)	0.9±1.6	0.6±0.9	0.8±0.8	0.8±1.1
	Trial 2 duration (sec) (mean ± SD)	19.3±17.1	18.6±15.4	18.8±14.6	22.3±17.0
	Failed to meet criterion	0/16 (0%)	0/16 (0%)	0/16 (0%)	0/16 (0%)
Session 2 (retention)	Trials to criterion	6.9±2.3	6.6±2.4	6.3±1.6	6.2±2.2
	Trial 1 errors (mean ± SD)	0.1±0.3	0.7±1.1	0.5±0.7	0.4±0.7
	Trial 1 duration (sec) (mean ± SD)	6.5±3.0	10.1±7.5	10.3±6.0	9.5±6.6
	Trial 2 errors (mean ± SD)	0.4±0.9	0.1±0.3	0.2±0.4	0.1±0.3
	Trial 2 duration (sec) (mean ± SD)	7.5±4.5	6.0±3.1	7.1±3.8	7.0±4.9
Females					
Session 1 (Learning)	Trials to criterion	7.5±2.5	7.8±2.9	7.5±2.9	7.3±3.1
	Trial 1 errors (mean ± SD)	0.9±0.7	0.8±0.7	0.5±1.0	0.7±0.9
	Trial 1 duration (sec) (mean ± SD)	17.2±13.4	14.3±8.7	16.4±15.3	15.7±9.0
	Trial 2 errors (mean ± SD)	0.7±1.1	0.6±0.9	0.9±1.0	0.5±1.0
	Trial 2 duration (sec) (mean ± SD)	14.9±12.3	12.8±10.0	14.5±8.8	11.6±14.0
	Failed to meet criterion	0/16 (0%)	0/16 (0%)	1/16 (6%)	1/16 (6%)
Session 2 (retention)	Trials to criterion	6.5±1.8	7.4±3.6	6.6±2.7	7.1±2.9
	Trial 1 errors (mean ± SD)	0.9±1.8	0.4±0.5	0.3±0.6	0.3±0.7
	Trial 1 duration (sec) (mean ± SD)	13.1±14.8	10.0±7.2	6.9±4.7	8.7±7.6
	Trial 2 errors (mean ± SD)	0.3±0.6	0.1±0.3	0.5±1.1	0.4±0.7
	Trial 2 duration (sec) (mean ± SD)	6.2±5.1	5.3±2.8	8.7±12.0	6.6±5.1

a Data obtained from Table 26, pages 234-236, MRID 45537501. N=16/sex/dose, except for session 2 females where N=15.
*p<0.05.

e. **Ophthalmology:** There were no treatment-related ocular effects in any treated animals compared to controls.

5. **Postmortem results:**

a. **Brain weights:** Mean brain weight data are presented in Table 14. The only statistically significant finding was an increase in brain weight of low dose males at termination. This finding was not considered treatment related. In conclusion, absolute and relative brain weights of male and female offspring were unaffected by treatment on PND 11 or at study termination.

TABLE 14. Mean (±SD) Brain Weight Data in Offspring [a]				
Parameter	Dose (ppm)			
	0	100	250	750
Males				
Day 11				
Terminal body weight (g)	21.5±2.9	22.9±2.4	21.7±2.9	19.3±2.3
Brain weight (g)	1.333±0.091	1.394±0.094	1.371±0.090	1.346±0.098
Brain-to-body weight ratio	6.292±0.875	6.121±0.459	6.380±0.614	7.025±0.745
Termination				
Terminal body weight (g)	307.4±25.4	316.2±25.9	311.6±32.6	296.3±21.8
Brain weight (g) (Fixed)	1.902±0.093	1.893±0.081	1.907±0.076	1.837±0.068
Brain weight (g) (Fresh)	1.908±0.115	1.943±0.078	1.913±0.103	1.897±0.112
Brain-to-body weight ratio	0.622±0.047	0.602±0.051	0.617±0.061	0.622±0.047
Females				
Day 11				
Terminal body weight (g)	21.6±1.9	21.2±2.2	21.9±2.4	19.3±2.4
Brain weight (g)	1.358±0.073	1.297±0.079	1.340±0.090	1.290±0.091
Brain-to-body weight ratio	6.337±0.669	6.142±0.347	6.165±0.398	6.752±0.571
Termination				
Terminal body weight (g)	204.4±11.0	195.6±12.9	197.5±13.6	197.8±12.2
Brain weight (g) (Fixed)	1.737±0.027	1.795*±0.059	1.744±0.051	1.742±0.048
Brain weight (g) (Fresh)	1.776±0.049	1.822±0.103	1.797±0.076	1.774±0.063
Brain-to-body weight ratio	0.852±0.053	0.922±0.071	0.886±0.054	0.883±0.039

a Data obtained from pages 952-953 & 955-956, 958-959 MRID 45537501. *p<0.05
N = 10/sex/dose; Fresh weights are from different rats.

C. NEUROPATHOLOGY

1. **Macroscopic examination:** No treatment-related effects were reported for male or female offspring at postnatal day 11 or study termination.

2. **Microscopic examination:** No significant treatment-related effects were noted on postnatal days 11 or 70.

   Brain morphometry: Data from anterior/posterior cerebral and cerebellar measurements and caudate/putamen widths are shown in Table 15. In high dose female animals at study termination, there was a statistically significant decrease in caudate/putamen thickness (3.7504 vs 3.6774 mm (-2%); p <0.03; t test). There was also a reduction in this brain region in PND 11 females, 2.77 vs 2.62 mm (-5.4%), but it was not statistically significant (p = 0.075). This finding did not correspond to changes in mean brain weights. Because changes were seen in this area in females both at PND 11 and at termination, this finding is considered as treatment related, and the registrant should examine mid dose tissue from both PND 11 and terminal females to establish an NOAEL. Additional microscopic examination of this region in the high and mid dose groups should also be considered to characterize this effect.

TABLE 15. Mean (±SD) morphometric data in offspring [a]				
Parameter	Dose (ppm)			
	0	100	250	750
Males				
Day 11				
Anterior to posterior cerebrum length (mm)	12.40±0.42	12.68±0.39	12.70±0.22	12.44±0.41
Anterior to posterior cerebellum length (mm)	6.49±0.40	6.52±0.41	6.52±0.50	6.50±0.54
Caudate/Putamen width	2.70±0.22			2.71±0.22
Termination				
Anterior to posterior cerebrum length (mm)	14.74±0.26	14.49±0.47	14.58±0.26	14.33±0.28
Anterior to posterior cerebellum length (mm)	8.27±0.42	7.94±0.41	8.29±0.31	8.09±0.27
Caudate/Putamen width	3.67±0.08			3.70±0.06
Females				
Day 11				
Anterior to posterior cerebrum length (mm)	12.53±0.36	12.29±0.37	12.54±0.30	12.16±0.43
Anterior to posterior cerebellum length (mm)	6.39±0.48	6.25±0.50	6.41±0.45	6.40±0.39
Caudate/Putamen width	2.77±0.14			2.62±0.23
Termination				
Anterior to posterior cerebrum length (mm)	14.36±0.26	14.35±0.23	14.22±0.26	14.24±0.28
Anterior to posterior cerebellum length (mm)	8.29±0.32	8.09±0.27	8.16±0.48	8.14±0.33
Caudate/Putamen width	3.75±0.08			3.68±0.06*

a Data obtained from pages 952-953 & 955-956, MRID 45537501.
N = 10/sex/dose; * $p<0.05$, t test, 2 tailed.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the overall NOEL is 250 ppm for dams and offspring based on decreased food consumption in dams and decreased body weight and activity in the offspring. There were no morphologic changes in neural tissues in offspring.

B. **REVIEWER COMMENTS:** Treatment-related effects for maternal animals were limited to decreased food consumption for dams in the 750 ppm group compared to controls during the third week of gestation (9%, n.s.) and first week of lactation (14%, $p<0.05$). There was also a slight decrease in body weight gain (67% of control levels, not statistically analyzed) during the first week of lactation. These effects are considered minimal, and a higher dose should have been used. However, given the treatment-related findings in pups at the high dose (see below), additional data will not be required at this time.

The maternal LOAEL for Imidacloprid in rats is 55-58 mg/kg/day in the diet based on decreased food consumption. The maternal NOAEL is 20 mg/kg/day in the diet.

Treatment-related effects in offspring were also limited to the high dose, and included decreased body weight in both sexes (9-13% during lactation and 3-11% after weaning). Body weights were similar across groups on PND0.

Motor activity was also decreased at the high dose only, in both sexes on PND 17 and in females on PND 21. The decreases in overall activity were not statistically significant, but were consistent in both sexes and are of sufficient magnitude (31-38%) to be considered adverse; this interpretation is supported by statistically significant decreases (47-48%) in females during the first interval on PND17.

No treatment-related effects were seen in FOB or in auditory startle, but increased latency to enter the dark chamber was seen in the first trial of the passive avoidance testing on PND24, for all treated males and for low and high dose females. This increase was not statistically significant or dose-related (low and high dose values were higher than mid dose values for both sexes), and the magnitude was larger in males (130-158% of control) than females (0-139% of control). Because the effect was seen prior to acquisition of the task, and no effects were seen on acquisition or retention-related parameters, the interpretation is unclear and the finding is not considered adverse. During water maze testing, an increase in both Trial 1 errors and duration was seen during session 1, in males only (errors were 400% of control levels, duration was 237% of control, $p<0.05$). The water maze findings are considered treatment-related and adverse.

In high dose female offspring at study termination, there was a statistically significant decrease in caudate/putamen thickness (3.7504 vs 3.6774 mm (-2%); $p <0.03$; t test). There was also a reduction in this brain region in PND 11 females, 2.77 vs 2.62 mm (-5.4%), but it was not statistically significant ($p = 0.075$). This finding did not correspond to changes in mean brain weights, but caudate/putamen is a small region of the brain. Our overall judgment is that it is prudent to consider the high dose changes in the caudate/putamen widths in females as treated related; they are statistically significant in the females at termination, and similar, though not statistically significant, decreases were seen in PND 11 females. This suggests a persistent change in these structures. Many arguments are often put forward as to why these or similar effects seen should be discounted, including only in one sex, not correlated with functional changes or other morphometric or microscopic pathology. Changes in one sex are possible and common in toxicology and not a basis for discounting effects. The clinical/pathological correlation in neurotoxicity studies is quite poor in general, so this should not be considered sufficient. Lack of changes in thickness of cell layers or cellular pathology provides detail that do contribute to the weight of evidence. But these measures are small samples of linear measurements of three dimensional structures, that only faintly approximately the volume of the tissue involved. In addition, there are many ways that tissue size could be adversely impacted in the absence of such changes, e.g. by disturbances in fluid balance. So when changes in these simple measures are seen that are not well understood, the sufficiency of the bases for discounting them, e.g., not correlated with other simple measures, are also weak approximations of the underlying processes related to them.

The offspring LOAEL for Imidacloprid in rats is 55-58 mg/kg/day in the diet, based on decreased body weight and body weight gain, decreased motor activity, and decreased caudate/putamen width in females. The offspring NOAEL is 20 mg/kg/day in the diet.

C. **STUDY DEFICIENCIES:** Registrant should submit: (1) complete analytical data; (2) morphometric measurements for caudate/putamen for females at intermediate dose levels; and (3) additional positive control data, as described above.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
129099	45537501	Dev Neurotox	Rats		Oral	Dict						Maternal
129099	45537501	Dev Neurotox	Rats		Oral	Diet						Offspring