DATA EVALUATION RECORD

ACETAMIPRID

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 46255619

Prepared for

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

Prepared by

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DATA EVALUATION RECORD

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

PC CODE: 099050 **DP BARCODE:** D303615
TXR#: 0052563

TEST MATERIAL (PURITY): Technical Grade Acetamiprid (>99%)

CITATION: Nemec, M (2003) An Oral Developmental Neurotoxicity Study in Rats. WIL Research Laboratories, Inc., Ashland, Ohio. Laboratory Project ID WIL-21193; November 21, 2003. MRID 46255619. Unpublished
See page 2 for additional 5 citations.

SPONSOR: Nippon Soda Co., Ltd., Chiyoda-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46255619), Acetamiprid (>99% a.i., lot # NNI-03) was administered to 25 mated female Crl:CD7(SD)IGS BR rats/dose by gavage at doses of 0, 2.5, 10 and 45 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 21 in a volume of 5 mL/kg body weight. A Functional Operational Battery (FOB) was performed on 10 dams/dose on GDs 6 and 12, and on LDs 4 and 7. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for FOB and assessment of motor activity, auditory startle reflex habituation, learning and memory, and neuropathology at study termination (day 72 of age). On postnatal day 11, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Pup physical development was assessed by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

In the dams, the only systemic toxicity observed was a 4-5% decrease in body weight and a 15% decrease in body weight gain during gestation only and at the highest tested dose only. **The maternal NOAEL is 10 mg/kg/day and the maternal LOAEL was 45 mg/kg/day based on decreased body weight and body weight gain during gestation only.**

Treatment-related effects in the offspring at the high dose (45 mg/kg/day) include decreased body weights and body weight gains in males and females post-weaning, decreased pre-weaning

survival (PND 0-1), and decreased maximum auditory startle response in males on PND 20 and PND 60. Treatment had no adverse effects on clinical signs, developmental landmarks, FOB, brain weight or brain morphology. There is low confidence in the motor activity data because of problems with the control data (i.e, the normal developmental pattern was not seen in control animals). Therefore, no conclusions could be made on motor activity evaluation. The maximum auditory startle response amplitude was decreased 27% (PND 20) and 40% (PND 60) at 10 mg/kg/day, and it was decreased 42% (PND 20) and 53% (PND 60) at 45 mg/kg/day; only the decreased maximum auditory startle response in the 45 mg/kg/day males (PND 20 and PND 60) was considered treatment related. No conclusions can be made on the effects of acetamiprid on learning and memory because of the high variability in the data.

The offspring LOAEL is 45 mg/kg/day based on decreased body weights and body weight gains in males and females, decreased pre-weaning survival (PND 0-1), and decreased maximum auditory startle response in males on PND 20 and PND 60. The offspring NOAEL is 10 mg/kg/day.

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

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

Additional Citations:

Nemec, M.; Beck, M.; Sloter, E. (2006) Rebuttal of Data Evaluation Record for Acetamiprid. WIL Research Laboratories, Inc. Laboratory Project ID WIL-21193; 2006. MRID 46779201. Unpublished

Nemec, M., (2004) A Dose Range-Finding Study for a Developmental Neurotoxicity Study of Acetamiprid in Rats. WIL Research Laboratories, Inc. Laboratory Project ID: WIL/21192. 2004. MRID 46779202. Unpublished

Schaefer, G., (2006) Validation of Developmental Neurotoxicity Endpoints in Rats Administered Methimazole in Drinking Water. WIL Research Laboratories, Inc. Laboratory. Project ID: WIL/99199. 2006. MRID 46779203. Unpublished

Pitt, J. (2006) A Validation Study for Developmental Neurotoxicity Endpoints.: Effect of Proylthiouracil (PTU) on Developmental Neurotoxicity Endpoints in Crl:CD (SD) IGS BR Rats. WIL Research Laboratories, Inc. Project ID: WIL-99126. 2006. MRID 46779204. Unpublished

Li, A.; Lau, E. (2007) Non-GLP Statistical Analysis Conducted by Exponent, Inc: Acetamiprid DNT Study. WIL Research Laboratories, Project ID: WIL-21193. 2007. MRID 46255619. Unpublished

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Technical grade Acetamiprid

Description:	Light brown crystalline powder
Lot #:	NNI-03
Purity:	>99 % a.i.
Compound Stability:	Stable for > 4 years at -20^{0}C (Appendix A, MRID 46255619)

2. Vehicle: 5% gum arabic (Spectrum Laboratory Products, New Brunswick, NJ), 0.01% polyoxyethylene-sorbitan monooleate (Tween 80) (Sigma Aldrich Company, St. Louis, MI) and deionized water

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD7(SD)IGS BR	
Age at study initiation:	74-78 days	
Wt. at study initiation:	226-265 g (GD 0)	
Source:	Charles River Laboratories, St. Constant, Quebec, Canada	
Housing:	Individually in wire-mesh cages (prior to mating for dams and for offspring used for developmental landmarks, neurobehavioral testing and neurobehavioral examination); plastic maternity cages with nesting material (post-mating) for dams and offspring	
Diet:	PMI Nutrition International, Inc. Certified Rodent LabDiet7 5002 , *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	68.4-72.5°F
	Humidity:	32.5-44.8%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Ten days	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: November 11, 1999; End: October 21, 2000
Morphometric analyses completed July 14, 2003

2. Study schedule: Crl:CD7(SD)IGS BR rats (25/dose group) were administered the test material by gavage from gestation day (GD) 6 through lactation day (LD) 21. On postnatal day (PND) 4, litters meeting the sex ratio criteria (4:4, 3:5 or 5:3, male: female) were standardized to 8 pups. Pups were weaned from the dam on PND 21; dams were sacrificed after weaning. There was no direct dosing of the pups; offspring were exposed to the test material *in utero* and through nursing during lactation. Pups remained on study up to PND 72.

3. **Mating procedure:** Females meeting acceptable body weight requirements (210-270 g) were placed in a cage with a resident male from the same strain and breeding source. The males were untreated, sexually mature rats used exclusively for breeding. The day that a vaginal plug or sperm on a vaginal smear was detected was designated GD 0.

4. **Animal assignment:** The mated females were randomly assigned to treatment groups using a computer program, as shown in Table 1. On GDs 6 and 12 and LDs 4 and 7, 10 females/group were examined outside the cage using a functional observation battery (FOB) of tests.

   Subsets of 10 pups/sex/group were assigned for detailed clinical observations (FOB), measurements of motor activity, auditory startle, learning and memory, and neuropathology as noted in Table 1.

TABLE 1. Study design[a]

Experimental parameter	Dose (mg/kg/day)			
	0	2.5	10	45
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	25	25	25	25
FOB (GDs 6 and 12, LDs 4 and 7)	10	10	10	10
Offspring				
	No. of offspring assigned			
Detailed clinical/FOB (PNDs 4, 11, 21, 34, 45, 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PNDs 13, 17, 21, 61)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PNDs 20, 60)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PNDs 22, 62)	10/sex	10/sex	10/sex	10/sex
Brain weight				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 72	10/sex	10/sex	10/sex	10/sex
Neuropathology				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 72	10/sex	10/sex	10/sex	10/sex

[a] Data obtained from page 29 of MRID 46255619.

5. **Dose selection rationale:** A preliminary dose range-finding study was conducted and submitted to the Agency (MRID 46779202.) In this study, 12 female Crl:CD (SD)IGS BR rats/group were fed 0, 5, 10, 20, or 40 mg/kg/day acetamiprid once daily via gavage from gestation day 6 through lactation day 10. The dose volume was 5 mL/kg; the vehicle was 5% Gum Arabic with 0.01% Tween 80 solution. All animals were observed twice daily for

appearance and behavior. All animals were allowed to deliver and rear their pups through weaning (lactation day 21).

All females survived to scheduled necropsy; no treatment-related clinical signs or internal findings were noted at any dose level. Eleven of twelve maternal animals in the control and 10 mg/kg/day groups delivered litters; all maternal animals in the other groups delivered litters. Maternal body weights were minimally decreased throughout gestation at 20 and 40 mg/kg/day; overall body weight gains were decreased 7.8% and 17%, respectively, at the end of gestation (GD 6-20). Food consumption was also decreased 12-29% throughout gestation in the 40 mg/kg/day dose group, with an overall decrease in food consumption of 17%. Body weight, body weight gains, and food consumption were unaffected throughout lactation, and all dose groups were similar to or greater than the controls in body weight, body weight gains, and food consumption at the end of lactation.

The mean numbers of pups born, mean live litter size, and sex ratio at birth were unaffected. Postnatal survival and overall health condition were unaffected by treatment.

6. **Dosage administration:** Acetamiprid was administered to maternal animals by gavage on GD 6 through LD 21, in a volume of 5 mL/kg of body weight, unless parturition was occurring at the time of dosing. Control animals received 5 mL/kg of the vehicle, 5% gum arabic with 0.01% Tween 80. Dosing was based on the most recent body weight determination.

7. **Dosage preparation and analysis:** The vehicle was prepared by mixing an appropriate amount of gum arabic with deionized water and Tween 80 to prepare a 5% solution. The solution was stored in the refrigerator for no more than 8 days. For the treated groups, the appropriate amount of test article was weighed into tared, calibrated storage containers. Approximately 70% of the vehicle was added and the mixture stirred. The mixture was then homogenized using a Silverson L4RT homogenizer to obtain a uniform solution. The remaining amount of vehicle was added to bring the volume to the calibration mark. The pH of the dosing solutions, including the control group, ranged from 4.71 to 5.04. The dosing formulations were stored in the refrigerator, protected from light. The study director visually inspected the solutions for homogeneity prior to dosing.

   Prior to dosing, representative batches of the dosing formulations from the 5 and 45 mg/kg/day groups were prepared. [Low dose was reduced to 2.5 mg/kg/day (0.5 mg/mL) prior to initiation of dosing.] Duplicate 10-mL samples from the middle stratum of the control formulation and from the top, middle and bottom of each test solution were collected. One set of samples was used to confirm homogeneity. The remaining samples from each dose level were combined and stored refrigerated for 8 days and then analyzed for stability. During the in-life phase, one 10-mL sample was collected from the middle stratum of each dosing solution to confirm the concentration of the formulations.

Results:

Homogeneity analysis: The concentration of samples from the top, middle, and bottom of the 0.5 mg/mL and 9 mg/mL formulations varied by less than 2% and 7%, respectively.

Stability analysis: After 8 days of refrigeration, the concentrations (% of initial concentration) for the 0.5 mg/mL (2.5 mg/kg/day) and 9 mg/mL (45 mg/kg/day) formulations were 0.568 mg/mL (103%) and 9.46 mg/mL (103%), respectively.

Concentration analysis: The mean concentrations (% of initial concentration) for the 0.5 mg/mL (2.5 mg/kg/day), 2 mg/mL (10 mg/kg/day) and 9 mg/mL (45 mg/kg/day) formulations were 0.507 mg/mL (101%), 2.0 mg/mL (99.9%) and 9.17 mg/mL (102%), respectively.

The analytical data indicated that the concentration, homogeneity, and stability of acetamiprid in the 5% gum arabic with 0.01% Tween 80 preparations were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Twice daily checks for mortality or moribundity were conducted on maternal animals. Detailed physical examinations of the dams were conducted daily, prior to treatment, through LD 21. Animals were observed daily for signs of toxicity for approximately one hour after dosing. Females were observed twice daily during the period of expected parturition for signs of dystocia, prolonged labor, delayed labor or other difficulties.

Ten randomly selected dams per group were observed outside the home cage at least twice during the gestation period (days 6 and 12) and twice during the lactation period (days 4 and 7). The size of the arena for the FOB observations was not provided. Females that were selected for FOB observations and that did not deliver were arbitrarily replaced by females that did deliver. The test period was one minute in duration. Testing was performed by the same technicians, when possible, who were blinded to the animal group. No other experimental details were given. The following functional observations were recorded.

Individual maternal body weight was recorded on GDs 0, 3, 6, 9, 12, 15 and 20. Females with litters were weighed on LDs 1, 4, 7, 10, 16, and 21. Food consumption measurements were recorded on GDs 0, 3, 6, 9, 12, 15 and 20 and on LDs 1, 4, 7, 10, 16 and 21. Food intake was calculated as g/animal/day and g/kg/day.

	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.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as PND 0. When parturition was complete, litters were sexed and examined for gross malformations. The numbers of stillborn and live pups were recorded. The duration of gestation was calculated using the date delivery began. Each litter was examined twice daily for survival and a daily record of litter size was maintained.

   On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). If a litter did not meet the sex ratio criteria (4:4, 3:5 or 5:3, male to female), the pups from the litter were euthanized.

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

3) **Postweaning observations:** After weaning on PND 21, offspring were examined weekly until euthanasia. Detailed clinical observations (FOB, as described below) were conducted on PNDs 34, 45 and 60; body weight was measured on these days.

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

i) **Functional observational battery (FOB):** On PNDs 4, 11, 21, 34, 45, and 60, ten offspring/sex/group were examined outside the home cage in a FOB assessment. The same parameters assessed in the maternal FOB were examined for offspring, as

appropriate for the developmental stage being observed. No other experimental details were given.

ii) **Motor activity testing:** Motor activity was evaluated in 10 pups/sex/dose on PNDs 13, 17, 21 and 61 using the SDI Photobeam Activity System. The same animals were tested at each interval, when possible. The system used a series of infrared photobeams surrounding a clear, plastic rectangular cage to quantify motor activity. Data were collected on each animal in five-minute epochs and for the test session duration of 60 minutes. Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of a single photobeam) and ambulatory motor activity (interruption of two or more consecutive photobeams).

iii) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on 10 offspring/sex/dose on PNDs 20 and 60, using the SR-Lab Startle Response System. The same animals were tested at each interval. Each isolation chamber measured 15 x 16 x 23 inches and was composed of a wood core covered with plastic laminate. Each cabinet was equipped with an internal light, a fan, two viewing lenses and a complete white-noise generation system set to operate at 70 decibels (db). The animal was placed in a cylindrical enclosure equipped with a motion sensor which was then placed into the isolation chamber. The animals were allowed a 5 minute acclimation period in the response chamber with 65-db broadband background white noise. The startle stimulus for each trial was 115-db mixed-frequency noise burst stimulus, approximately 20 milliseconds in duration. Responses were recorded during the first 100 milliseconds of the startle stimulus. Each test session consisted of 50 trials, with an 8-second interval. Maximum response amplitude (V_{MAX}), average response amplitude (V_{AVE}) and latency to V_{MAX} (T_{MAX}) were recorded.

iv) **Learning and memory testing:** Learning and memory testing was performed in 10 offspring/sex/dose using a water-filled, eight-unit T-maze similar to that described by Biel[1]. Animals were required to traverse the maze and escape by locating a platform hidden beneath the water surface. The time required to traverse the maze and the number of errors were recorded; an error was defined as any instance when an animal deviated from the correct channel with all four feet. The testing intervals were on PNDs 22 and 62; the same animals were not tested on each day. The testing intervals consisted of three phases conducted over seven consecutive days. For phase one, which was performed on day one of the Biel maze procedure, animals were placed in a straight channel opposite the escape platform and the time required for each animal to escape was recorded. Each animal was given four trials to assess swimming ability and motivation.

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

In phase two, which was conducted on days 2-6 of the Biel maze procedure, animals were allowed two trials per day for two consecutive days to solve the maze in path B (reverse of path A) to test sequential learning. (Note: Path A was not identified in the study report; it is assumed to be the path used for phase one.) For each trial, an animal was allowed three minutes to solve the maze; it was removed at the end of the allotted time if it was not successful. The minimum intertrial interval was one hour.

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

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

2. **Postmortem observations**:

a. **Maternal animals:** Unscheduled deaths were subjected to a gross necropsy. The number and location of implantation sites and corpora lutea were recorded; recognizable fetuses were examined externally and discarded. Tissues were preserved if deemed necessary by gross findings and the carcass was discarded.

Females that failed to deliver within 25 days after mating were euthanized with carbon dioxide inhalation. The abdominal and thoracic cavities were examined and the number of implantation sites recorded. Uteri with no external evidence of implantation sites were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. Tissues were preserved if deemed necessary by gross findings and the carcass was discarded.

Females with total litter loss were euthanized within 24 hours by carbon dioxide inhalation. Females that failed to meet the sex ratio criteria (4:4, 3:5 or 5:3, male:female) were euthanized on LD 4. Females that delivered were euthanized on LD 21. All these dams were subjected to gross necropsy and the procedures described above for females that failed to deliver.

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

On PND 11, one male and one female from each litter were euthanized by carbon dioxide and perfused *in situ* (fixative not specified) for macroscopic neurological examination. Of these pups, 10 randomly selected pups/sex/group from all dose groups were prepared for microscopic neuropathologic examination; however, only the control and high dose groups were evaluated. The brains (including olfactory bulbs) were weighed and the size (length and width) recorded, along with any abnormal coloration or lesions of the brain and spinal cord. The brains were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Sections of the olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum were examined.

On PND 72, a minimum of ten pups/sex/group randomly selected from those pups selected for motor activity, auditory startle and learning and memory tests were euthanized by carbon dioxide inhalation and perfused *in situ* (fixative not specified). The brains (including olfactory bulbs) were weighed and the size (length and width) recorded, along with any abnormal coloration or lesions of the brain and spinal cord. The tissues listed below from the control and high dose animals were examined microscopically. The central nervous system and peripheral nerve system tissues were embedded in paraffin and plastic, respectively.

Brain-olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum
Spinal cord - at cervical swellings C_3-C_7 and at lumbar swellings T_{13}-T_{14}
Gasserian ganglion/trigeminal nerves (2)
Lumbar dorsal root galglion at T_{13}-T_{14}*
Lumbar dorsal root fibers at T_{13}-T_{14}*
Lumbar ventral root fibers at T_{13}-T_{14}*
Cervical dorsal root ganglion at C_3-C_7*
Cervical ventral root fibers at C_3-C_7*
Sciatic nerves (2)
Sural nerves (2)
Tibial nerves (2)
Peroneal nerves (2)
Optic nerves
Eyes
Skeletal muscle (gastrocnemius)

* = 4-6 tissues were collected at necropsy; 2 tissues were evaluated microscopically
Brain morphometric measurements were performed on PNDs 11 and 72. Quantitative examinations of the brains were done on images digitized from the glass slides using Pax-ItJ computer software. Linear measurements of the brain made bilaterally were designated as Levels 1, 3 and 5. Level 1 was a coronal section corresponding to Plate 11 in the Paxinos and Watson rat brain atlas[2]. The two measurements taken bilaterally on

[2] Paxinos, G. and Watson, C (1998) The Rat Brain in Stereotoxic Coordinates, Academic Press, New York.

Level 1 consisted of the height of a hemisphere (Ht Hemisphere) measured just at the beginning of the lateral ventricle and the vertical cortical thickness (V Thickness Cortex) measured at the apex of the corpus callosum (the 12 o=clock position) and parallel to the height. Level 3 was a coronal section corresponding to Plate 21 of the atlas. The four measurements on Level 3 included: 1) the radial thickness (Radial Thickness Cortex) of the frontoparietal cortex (at the 2 o=clock position on the hemisphere on the right in the image and at the 10 o=clock position of the hemisphere on the left); 2) the vertical height between the layers of the hippocampal pyramidal neurons (V Ht Btw Hippocampal Pyrimidal Neuron Layer) measured along a line that passed through the termination of the dorsal limb of the medial dentate hilus; 3) the vertical height of the dentate hilus (V Ht Dentate Hilus) measured at the termination of the ventral limb; and 4) the length of the ventral limb of the dentate hilus (Length Ventral Limb Dentate Hilus). Level 5 measurements were single from one midsagittal section. Level 5, corresponding to Plate 44 of the atlas, measured the thickness of the caudal pons (V Thickness of Pons) toward lobule no. 9 and the distance across the base of cerebellar lobule no. 9 (Base of Lobule 9) measured perpendicular to the white matter tract. Measurements were made on homologous sections to ensure that the dimensions of the regions were comparable. If needed, pairwise matching between measured groups was performed to achieve comparable homology.

D. DATA ANALYSIS:

1. **Statistical analyses:** Analyses were conducted for a minimum significance level of 5% comparing each treated group to the control group with two-tailed group comparisons. The following parameters were analyzed using the Analysis of Variance (ANOVA) (two-tailed) with Dunnett's test: maternal gestation and lactation body weights and body weight gains, maternal food consumption, mean litter weight, length of gestation, number of pups born, live litter size, organ weight, motor activity, startle response, age at balanopreputial separation or vaginal patency, Biel maze and morphometric analyses.

   Kruskal-Wallis test with Mann-Whitney U test was used to analyze pup sex at birth (% male per litter) and postnatal survival. The Kolmogorov-Smirnov test was used to analyze histopathologic findings.

2. **Indices:**

   a. **Reproductive indices:** The study report provided no formulas for calculating reproductive indices.

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

   $$\text{Mean Live Litter Size} = \frac{\text{Total number of viable pups on PND 0}}{\text{No. of litters with viable pups PND 0}}$$

$$\text{Postnatal Survival Between Birth and PND 0 or PND 4 (\% per litter) (Live Birth Index)} = \frac{\sum (\text{No. of pups born alive per litter/ Total no. of pups born per litter})}{\text{No. of litters per group}} \times 100$$

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

Where N= PND 0-1, 1-4, 4-7, 7-14, 14-21, birth-4, 4-21

3. **Positive and historical control data:** Positive control data were not presented and no references to such data were given in the report. However, positive control data were submitted separately at a later date (March 7, 2006; MRID 46779201) and will be evaluated separately. WIL reproductive historical control data were included in the study report (Appendix C, MRID 46255619). Data on parental and neonatal observations were provided from 32 studies conducted from 1996-2000 with differing routes of administration and vehicles. The type of study performed was not identified. Auditory startle response data for measurements on PNDs 20 and 60 were provided from 9 studies; the time period when the studies were conducted was not identified. Motor activity historical control data from 2-6 studies for measurements on PNDs 13, 17, 21 and 60 (±1) were included. Brain morphometric historical control data from 4 studies were provided for various tissue levels and measurements on PNDs 11, 21, 22 and 72 in a limited number of animals (1-2/sex).

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** One female at 45 mg/kg/day died during parturition on GD 23. The animal had no significant clinical signs of toxicity prior to death. No internal findings were observed on gross necropsy.

   The incidences of hair loss and dried red material on the forelimbs, scabbing on the forelimbs and red material around the nose were increased in the treated groups. Salivation prior to dosing was increased in females at 45 mg/kg/day. The onset of these signs occurred during gestation. All animals in the 45 mg/kg/day group and nine animals at 10 mg/kg/day were observed to wipe their mouths on the cage bedding following dosing. The animals burrowed their faces into the bedding for approximately 30-45 seconds immediately after being returned to their cages. No treated-related findings were observed during the FOB observations on GDs 6 and 12 and LDs 4 and 7. The clinical observation results are summarized in Table 2.

TABLE 2. Clinical observations (total occurrence/number of animals affected)[a]				
Observation	Dose (mg/kg/day)			
	0	2.5	10	45
Hair loss, right forelimb	45/4	94/6	114/5	192/10
Hair loss, left forelimb	44/3	112/7	114/5	198/8
Dried red material, right forelimb	3/2	4/3	0/0	8/6
Dried red material, left forelimb	3/2	4/3	0/0	6/6
Scabbing, right forelimb	0/0	4/2	7/2	24/4
Scabbing, left forelimb	2/1	6/2	7/2	32/5
Dried red material around nose	3/2	8/5	4/3	13/9
Wipes mouth in bedding following dosing	0/0	0/0	15/9	513/25
Salivation prior to dosing	0/0	0/0	0/0	8/4

[a] Data obtained from pages 85-87, MRID 46255619.

2. **Body weight and food consumption**: Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 3. Mean body weight was significantly decreased (95-96% of control value) from GDs 9-20 in females at 45 mg/kg/day. A mean body weight loss of 3 g was observed in dams at 45 mg/kg/day during GDs 6-9 compared to a 13 g gain in control animals. During the entire gestation period (GDs 6-20), mean body weight gain was significantly decreased (85% of control value) in females at 45 mg/kg/day. On LD 1, mean body weight was significantly decreased (93% of control value) in females at 45 mg/kg/day but was comparable to controls during the remainder of lactation. Mean body weight gain was significantly increased for LDs 1-4 (150% of control value) and for LDs 1-21 (142% of control value), in high dose dams.

   Mean food consumption, evaluated as g/animal/day and g/kg/day, in females at 45 mg/kg/day was significantly decreased (60% of control value) during GDs 6-9 and 9-12 and for the entire gestation period (GDs 6-20) (82% of control value). During lactation, mean food consumption in treated animals was comparable to control intake.

   The body weight decreases observed in the dams during gestation (GD 9-20) were between 4-5% and were statistically significant. Although the dams recovered during lactation, this decrease in body weight, coupled with the decrease in body weight gain during the same time period, is considered an adverse effect[3].

3 The original DER concluded that the maternal body weight decrease was not an effect. However, HED has reconsidered given the rationale presented in the registrant's rebuttal (MRID 46779201).

TABLE 3. Selected mean (±SD) maternal body weight, body weight gain and food consumption[a]

Observations/study interval	Dose (mg/kg/day)			
	0	2.5	10	45
Gestation (n= 23-25)				
Body wt. Gestation day 0 (g)	247 ± 8.5	246 ± 8.3	247 ± 9.8	246 ± 9.6
Body wt. Gestation day 9 (g)	288 ± 10.7	287 ± 13.6	286 ± 10.5	275** ± 11.4 (95)
Body wt. Gestation day 15 (g)	320±15.9	321±16.4	320±15.4	304**±13.5 (95)
Body wt. Gestation day 20(g)	385±19.0	387±19.0	388±20.6	370*±18.7 (96)
Wt. gain gestation days 6-9 (g)	13±3.8	12±6.8	11±4.7	-3**±7.3
Wt. gain gestation days 6-20 (g)	110±12.3	112±16.0	112±14.2	93**±13.5 (85)
Food consumption gestation days 6-9 (g/animal/day)	20±1.8	20±1.9	19±1.6	12**±2.4 (60)
Food consumption gestation days 9-12 (g/animal/day)	20±1.8	20±1.9	19±1.6	12**±2.4 (60)
Food consumption gestation days 6-20 (g/animal/day	22±1.9	21±1.6	21±1.5	18**±1.5 (82)
Lactation (n=21-24)				
Body wt. lactation day 1 (g)	284±14.4	288±15.2	285±14.8	265**±17.4 (93)
Body wt. lactation day 10 (g)	325±17.6	324±16.4	332±19.8	318±12.6
Body wt. lactation day 21 (g)	317±15.0	317±14.5	321±17.4	313±14.5
Wt gain lactation days 1-4 (g)	18±7.8	19±5.3	23±11.1	27**±7.9 (150)
Wt gain lactation days 1-21(g)	33±11.9	31±14.1	34±12.7	47**±12.0 (142)
Food consumption lactation days 1-21 (g/animal/day)	50±4.7	49±4.2	52±3.7	50±3.5

[a]Data obtained from pages 105-112, MRID 46255619
* Statistically significantly different from control, $p<0.05$
** Statistically significantly different from control, $p< 0.01$.
Number in parentheses is % of control value, calculated by reviewer.

3. **Reproductive performance**: Two females each in the control, 2.5 and 10 mg/kg/day groups did not deliver offspring and were considered nongravid. The pregnancy rates in the 0, 2.5, 10 and 45 mg/kg/day groups were 92%, 92%, 92% and 100%, respectively. Mean gestation length in the respective groups was 21.5, 21.5, 21.5 and 21.7 days. No other reproductive indices were calculated. Results for the maternal animals are summarized in Table 4.

TABLE 4. Reproductive performance[a]				
	Dose (mg/kg/day)			
Observation	**0**	**2.5**	**10**	**45**
Number mated	25	25	25	25
Number pregnant	23	23	23	25[b]
Fertility index (%)	92	92	92	100[b]
Intercurrent deaths	0	0	0	1
Mean (±SD) gestation duration (days)	21.5 ± 0.51	21.5 ± 0.51	21.5 ± 0.59	21.7 ± 0.63[b]
Incidence of dystocia	0	0	0	1

[a] Data obtained from pages 84 and 113, MRID 46255619.
[b] One dam which died during parturition is included in calculation.

B. OFFSPRING:

1. Viability and clinical signs: Litter size and viability (survival) results for pups during lactation are summarized in Table 5. The mean number of pups born/dam, number of pups born and the percentage of males per litter at birth were not affected by treatment. Live litter size (PND 0; n.s.) and postnatal survival on PNDs 0-1 ($p<0.05$) were decreased at 45 mg/kg/day; three dams at 45 mg/kg/day had total litter loss on PND 1. This decreased pre-weaning survival on PND 0-1 is considered treatment-related. Survival was non-significantly reduced in offspring from dams at 45 mg/kg/day for birth to PND 4 and was similar to controls for the period PND 4-21. Postnatal survival in the treated groups was similar to controls for the remainder of the pre-weaning intervals (PND 1-4, 4-7, 7-14 and 14-21). The total number of pups found dead or euthanized *in extremis* or due to death of the dam during the pre-weaning period was 7, 14, 3 and 39 in the 0, 2.5, 10 and 45 mg/kg/day groups, respectively. The number of pups missing and presumed cannibalized was 2, 5, 3 and 22 in the respective groups.

   All remaining pups survived to their respective scheduled necropsies during the post-weaning period. The percentage of males on the day of birth was non-significantly decreased in the 45 mg/kg/day group (47.3 vs 52.9 in controls) but there was no dose-response effect. There were no treatment-related clinical signs of toxicity in the offspring.

TABLE 5. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	0	2.5	10	45
Total number born[b]	348	353	357	371[c]
Pups/dam delivered	15.1±2.1	15.3±3.1	15.5±2.1	15.5±1.7
Number of litters	23	23	23	24[c]
Number with live born litters	23	23	23	24[c]
Number with stillborn pups[b]	3	2	2	6
Number born live	345	351	355	346
Live litter size (PND 0)	15.0±2.1	15.3±3.2	15.4±2.1	14.4±3.4[c]
Number born dead	3	2	2	26
Sex Ratio Day 0 (% %)	52.9±16.1	51.3±9.1	54.4±11.2	47.3±16.1
# Deaths Pre-weaning[d]				
Found dead	7	14	3	37
Euthanized *in extremis*	0	0	0	2
Missing	2	5	3	22
Total	9	19	6	61
Percentage of litter survival:				
PND 0 (relative to number born)	99.1±2.3	99.4±1.9	99.5±1.8	94.4±19.4[c]
PND 0 - PND 1	99.0±2.3	96.7±9.2	99.4±1.9	85.1*±33.1
PND 1 - PND 4 (pre-selection)	100.0±0.0	99.1±2.5	99.8±1.2	99.7±1.5
PND 4 (post-selection) - PND 7	99.4±2.7	100.0±0.0	99.4±2.7	100.0±0.0
PND 7 - PND 14	100.0±0.0	100.0±0.0	100.0±0.0	99.2±3.6
PND 14 - PND 21	99.2±3.6	100.0±0.0	100.0±0.0	100.0±0.0
Birth - PND 4 (pre-selection)	98.1±3.0	95.4±10.1	98.7±2.6	84.4±33.0[c]
PND 4 (post-selection) - PND 21	94.2±21.1	95.7±20.9	94.9±21.0	99.2±3.6
Live birth index[e]	99.1	99.4	99.4	93.3

[a] Data obtained from pages 115-117 in the study report, MRID 46255619.
[b] Calculated by the reviewer from data on pages 492-495, MRID 46255619.
[c] Does not include offspring from animal 33635 which died during parturition.
[d] Calculated by the reviewer from data on page 123 in the study report, MRID 46255619.
[e] Calculated by reviewer using formula: Live birth index = (# live born pups at birth/# pups born) x 100
* Statistically different from control, $p<0.05$

2. **Body weight:** On PND 1, mean body weight of offspring at 45 mg/kg/day was significantly decreased in females and slightly decreased in males. Mean body weight gain in the treated groups was comparable to the controls during the pre-weaning period. Selected mean preweaning pup body weight data are presented in Table 6.

TABLE 6. Selected mean (±SD) pre-weaning pup body weight and body weight gain [a]								
PND	Dose (mg/kg/day)							
	0	2.5	10	45	0	2.5	10	45
	Males				Females			
	Body weight (g)							
1	6.8±0.6	6.8±0.8	6.8±0.7	6.3±0.7 (93)	6.6±0.6	6.4±0.8	6.4±0.7	6.0*±0.7 (91)
4[b]	9.2±1.2	9.4±1.5	9.3±1.0	8.7±1.1 (95)	8.9±1.2	8.9±1.4	8.9±1.0	8.2±1.0 (92)
7	14.9±1.8	15.0±2.3	15.4±1.6	14.5±1.8	14.2±1.9	14.1±2.1	14.7±1.4	13.7±1.6
11	24.0±2.6	24.1±3.4	24.9±2.0	23.2±2.6	23.0±2.8	23.0±3.0	24.1±1.9	22.2±2.3
17	42.0±4.3	41.7±4.5	43.1±3.1	39.7±3.1	40.2±3.9	39.9±4.2	41.4±2.6	38.5±3.2
21	54.2±6.1	53.8±6.1	56.2±4.3	51.4±5.0	52.2±5.8	51.4±5.6	54.2±3.6	50.1±5.1
	Body Weight Gain (g)							
1-4 (pre-selection)	2.3±0.7	2.5±0.8	2.5±0.5	2.4±0.5	2.3±0.7	2.5±0.8	2.5±0.5	2.3±0.5
4-7	5.7±1.1	5.8±1.3	6.1±0.7	5.8±0.9	5.2±1.2	5.4±1.3	5.8±0.6	5.5±0.8
17-21	12.2±2.4	12.1±2.6	13.1±2.6	11.7±2.6	12.1±2.5	11.5±2.4	12.8±2.3	11.6±2.5
1-21[c]	47.4	47.0	49.4	45.1	45.6	45	47.8	44.1

PND = post-natal day; N=21-23
[a] Data obtained from 176-178 and 182-183, MRID 46255619.
[b] Before standardization (culling).
[c] Calculated by the reviewer; no standard deviaton calculated.
* Statistically significantly different from control, $p < 0.05$
Number in parentheses is % of control value, calculated by reviewer.

Mean body weight at 45 mg/kg/day was significantly decreased in males (93-95% of control value) on PNDs 28, 49, 56, 63, 70 and 72 and in females (90-95% of control value) on PNDs 35, 42, 49, 56, 63, 70 and 72. Mean body weight gain at 45 mg/kg/day was significantly decreased in males (79-91% of control value) on PNDs 35-42, 42-49, 49-56 and 70-72 and in females (78-88% of control value) on PNDs 28-35 and 49-56; this decrease in post-weaning body weight and body weight gains in both males and females in the high dose group is considered treatment related. Mean body weight gain was also significantly decreased in males at 2.5 mg/kg/day (75-94% of control value) on PNDs 42-49, 49-56 and 70-72 and 10 mg/kg/day (83% of control value) on PNDs 70-72. Mean post-weaning offspring body weight data are presented in Table 7.

TABLE 7. Mean (±SD) post-weaning pup body weight and body weight gain (g) [a]								
PND	Dose (mg/kg/day)							
	0	2.5	10	45	0	2.5	10	45
	Males				Females			
	Body Weight (g)							
28	89.6±10.2	88.6±9.8	90.8±7.7	85.5*±7.8 (95)	82.7±7.7	81.4±8.8	83.8±6.3	79.9±5.5
35	144.6± 22.8	144.0± 15.5	150.5± 11.3	140.5± 10.7	128.7± 10.1	125.2± 12.9	125.9 ±12.0	115.8**± 12.2 (90)
42	208.2± 24.8	204.8± 19.9	213.5± 14.5	199.6± 15.9	164.6± 13.3	159.8± 14.4	160.6± 11.3	153.6**± 10.0 (93)
49	273.1± 28.7	265.5± 24.8	277.1± 18.4	258.6** ±21.0 (95)	192.6± 13.8	187.0± 17.2	189.5± 13.3	182.0**± 11.4 (94)
56	336.5± 32.5	324.8± 29.6	339.7± 21.5	315.2**± 24.9 (94)	218.8± 16.6	210.7*± 19.5	215.3± 16.5	205.1**± 13.4 (94)
63	380.5± 33.3	369.0± 33.0	387.9± 24.5	354.8**± 31.4 (93)	236.9± 19.0	228.9± 22.2	234.1± 17.5	223.1**± 14.6 (94)
70	418.0± 34.1	404.4± 35.5	424.0± 29.7	388.9**± 34.8 (93)	253.5± 19.7	244.6± 22.4	252.3± 19.0	239.6**± 16.3 (95)
72	432.9± 34.8	415.6*± 37.2	436.3± 30.1	400.7**± 36.1 (93)	258.0± 21.0	249.7± 24.7	255.8± 19.7	243.0**± 17.0 (94)
	Body Weight Gain (g)							
21-28	35.4±4.9	35.0±4.1	34.6±4.0	34.0±4.0	30.2±3.5	30.0±4.5	29.5±3.4	29.8±3.3
28-35	54.7±15.0	54.7±11.1	59.0±5.7	54.9±6.0	45.7±4.7	43.8±5.3	42.6±9.0	35.8**± 11.0 (78)
35-42	63.7±9.3	60.7±9.5	63.0±6.2	59.1*±6.9 (93)	35.9±8.2	34.6±4.6	34.7±7.1	37.8±9.1
42-49	64.9±9.3	60.7*±8.7 (94)	63.6±6.4	59.0**±7.0 (91)	28.0±8.2	27.2±5.7	28.9±4.8	28.4±5.6
49-56	63.4±7.1	59.2**±7.9 (93)	62.6±5.9	56.6**±6.4 (89)	26.2±5.9	23.7±5.4	25.8±5.7	23.1*±5.4 (88)
70-72	14.9±3.9	11.2**±5.4 (75)	12.3**±4.1 (83)	11.8**±4.2 (79)	4.5±6.1	5.1±6.1	3.4±5.1	3.4±6.7

PND = post-natal day; N=49-68
[a] Data obtained from 179-181 and 184-186, MRID 46255619.
* Statistically significantly different from control, $p < 0.05$
** Statistically significantly different from control, $p<0.01$
Number in parentheses is % of control value, calculated by reviewer.

3. Developmental landmarks:

a. **Sexual maturation:** The mean age of preputial separation in males was 43.6, 43.7, 43.1 and 43.9 days for the control, 2.5, 10 and 45 mg/kg/day groups, respectively. The mean age of vaginal opening in females was 32.2, 32.3, 32.7 and 32.5 days for the respective groups. Body weight at attainment for males was similar between the treated and control

groups; however, body weight in females was significantly decreased (94% of control value) at 45 mg/kg/day. The data are presented in Table 8.

Table 8. Mean (±SD) age (days) and body weight (g) at sexual maturation [a]

Parameter	Dose (mg/kg/day)			
	0	2.5	10	45
N (M/F)	22/22	21/22	22/22	21/21
Preputial separation				
age	43.6±1.0	43.7±1.6	43.1±1.5	43.9±1.8
body weight	226.3±18.7	223.1±15.7	223.4±11.1	215.8±12.1
Vaginal opening				
age	32.2±0.7	32.3±1.0	32.7±1.4	32.5±1.1
body weight	107.8±6.9	106.4±10.9	110.5±8.6	101.0*±7.0 (94)

[a] Data obtained from pages 190 and 193, MRID 46255619.
* Statistically significantly different from control, $p < 0.05$
Number in parentheses is % of control value, calculated by reviewer.

4. **Behavioral assessment:**

   a. **Functional observational battery**: There were no treatment-related FOB findings on PNDs 4, 11, 21, 35 or 60.

   b. **Motor/locomotor activity:** Total and ambulatory data are presented in Table 9. There is low confidence in the motor activity data because of problems with the control data. Measurements in PND 13 male controls appear high, and the normal developmental pattern was not seen in control males. Control values in PND 17 males and PND 13/17 females appear inconsistent (e.g., activity in PND 17 females was double that of PND 17 males).

   c. **Auditory startle reflex habituation :** The amplitude and latency data are presented in Table 10. The maximum response amplitude (V_{MAX}) and average response amplitude (V_{AVE}) were decreased in treated males in a dose-responsive manner on PNDs 20 and 60; the only significant decreases were in V_{AVE} (58% of control value) on PND 20 and V_{MAX} (47% of control value) and V_{AVE} (46% of control value) on PND 60 in males at 45 mg/kg/day. On PND 20, V_{MAX} and V_{AVE} were non-significantly reduced in females at 10 mg/kg/day (87-89% of control value) and 45 mg/kg/day (69-71% of control value). Latency to maximum response amplitude (T_{MAX}) in treated animals was similar to controls.

TABLE 9. Mean motor activity counts (mean ± SD) [a]

Test Day	Dose (mg/kg/day)			
	0	2.5	10	45
Males				
PND 13				
Total	580±396.2	293±175.9	380±236.0	371±179.9
Ambulatory	190±323.4	46±53.1	119±121.6	58±43.6
PND 17				
Total	442±332.4	705±854.9	766±683.5	696±692.2
Ambulatory	167±129.6	338±546.6	320±345.8	331±446.3
PND 21				
Total	416±180.2	631±471.1	407±145.3	458±197.6
Ambulatory	143±78.5	224±205.4	131±57.5	148±94.2
PND 61				
Total	1710±636.4	1906±467.2	1797±717.3	1680±626.4
Ambulatory	501±276.0	618±229.2	547±309.4	491±259.5
Females				
PND 13				
Total	288±222.4	465±439.6	397±182.5	319±217.9
Ambulatory	65±116.7	129±291.6	78±122.5	63±87.6
PND 17				
Total	815±530.1	581±354.8	453±482.5	984±670.0
Ambulatory	326±252.9	233±189.0	161±212.1	477±464.3
PND 21				
Total	423±248.7	373±206.3	386±264.5	502±210.8
Ambulatory	119±101.3	113±54.8	126±87.2	160±99.9
PND 61				
Total	1784±624.5	1513±596.3	1312±691.5	1450±559.7
Ambulatory	608±220.6	542±288.7	462±279.7	523±271.1

[a] Data obtained from pages 200-201, MRID 46255619
N = 10

For males at 45 mg/kg/day V_{MAX} and V_{AVE} values were below the range of historical control values (136.2-223.6 mv and 30.8-47.2 mv, respectively) on PND 20 and close to the lower end of the range for historical control values on PND 60 (87.2-247.1 mv and 21.2-52.9 mv, respectively). The control male group had V_{MAX} and V_{AVE} values that were at the high end of the historical control range and that were higher than the historical means on both testing days. For females at 45 mg/kg/day V_{MAX} and V_{AVE} values were below the range of historical control values (149.0-236.0 mv and 31.5-49.0 mv, respectively) on PND 20. V_{MAX} and V_{AVE} values for control females were within the range of historical control values and similar to the historical mean values on both days.

Nevertheless, for males, maximum auditory startle response amplitude was decreased 27% (PND 20) and 40% (PND 60) at 10 mg/kg/day, and it was decreased 42% (PND 20) and

53% (PND 60) at 45 mg/kg/day. Decreases observed at 2.5 mg/kg/day on PND 20 (15%) and PND 60 (10%) were not considered to be toxicologically significant.

TABLE 10. Auditory startle data (mean ± SD)[a]

	Dose (mg/kg/day)			
	0	2.5	10	45
	Males			
PND 20				
V_{MAX} (mv)	214.3 ± 94.9	182.7±84.6 (85)	157.3±61.7 (73)	123.4±57.3 (58)
T_{MAX} (msec)	24.9±3.6	24.1±3.4	25.4±3.5	24.3±3.1
V_{AVE} (mv)	45.5±18.6	39.0±16.9 (86)	33.8±11.0 (74)	26.6*±11.6 (58)
PND 60				
V_{MAX} (mv)	209.9±129.0	189.2±76.0 (90)	126.0±81.5 (60)	98.6*±66.6 (47)
T_{MAX} (msec)	31.5±4.8	29.3±5.5	32.9±6.3	33.6±5.3
V_{AVE} (mv)	46.5±28.0	40.3±16.6 (87)	29.0±19.2 (62)	21.6*±14.4 (46)
	Females			
PND 20				
V_{MAX} (mv)	181.2±50.8	182.5±54.1	160.6±47.9 (89)	129.0±56.6 (71)
T_{MAX} (msec)	22.7±2.6	24.0±2.6	24.6±4.6	25.3±2.0
V_{AVE} (mv)	39.5±10.0	37.9±10.5	34.4±10.9 (87)	27.4±12.5 (69)
PND 60				
V_{MAX} (mv)	78.2±36.1	78.7±30.7	79.6±40.4	80.1±37.0
T_{MAX} (msec)	34.3±4.4	32.9±4.7	33.9±4.5	30.9±4.7
V_{AVE} (mv)	16.7±7.6	17.3±7.8	16.2±7.0	16.5±6.7

[a]Data obtained from 194-195, MRID 46255619.
V_{MAX} = maximum response amplitude; T_{MAX} = latency to V_{MAX}; V_{AVE} = average response amplitude
N = 9-10
* Statistically significantly different from control value, $p<0.05$
Number in parentheses is % of control value, calculated by reviewer.

The registrant submitted two separate rebuttals to this conclusion (MRID 46779201 dated 3/7/06 and MRID 47181101 dated 4/18/07) stating that the LOAEL was 45 mg/kg/day, based on changes in maximum auditory startle measurements in postnatal day (PND) 20 males and females and in PND 60 males. To support their conclusion, the Registrant provided further statistical analyses of the auditory startle data consisting of a repeated measures analysis of variance (RANOVA). (See DP328052, TXR No. 0054508 for response to rebuttal).

The DNT Workgroup, along with Kevin Crofton of EPA's Office of Research and Development, met on three separate occasions on this matter (November 29, 2006, April 17th, 2007; and May 1st, 2007) to reconsider the biological and statistical significance of the data, using the additional statistical analyses provided by the Registrant (the BioSTAT analysis in 2006 and the Exponent analysis in 2007) together with an analysis conducted by the Chemistry and Exposure Branch (CEB) from HED (see CEB memo D341525). See Appendix I.

Details of the response to the registrant rebuttal, the various statistical analyses performed by the EPA, are presented in the CEB memo cited above and another detailed memo from the developmental neurotoxicity committee (TXR#0054508).

The new EPA statistical analysis supports the conclusion that there is a significant decrease in maximum auditory startle response in male rat pups at the mid-dose level of 10 mg/kg/day only when data from males at females at the two different time points (PND 20 and PND 60) are combined. However, there was no consensus within the Agency regarding the biological appropriateness of combining time point data due to different calibrations of the auditory startle equipment to account for heavier body weights in the pups at PND 60. Details are presented in the cited memos

Historical control data was provided by the registrant. Historical control data was from the conducting laboratory for the 5 years which bracket the acetamiprid DNT study (1999-2004, MRID 46779203, and 46779204). Review of the historical control data indicates that in general, the control means from the 1999 acetamiprid study are the highest of the control data presented. Even though the mean response of the PND 20 males from the 1999 acetamiprid study is relatively high, it is within the historical range of the other two studies conducted in 1999. The study response mean from the males of the 45 mg/kg/day dose group is lower than all the historical control means, except for one other study (i.e, study 20, 2001) this effect is considered treatment related. The graphs of the historical control data along with the control data from the acetamiprid DNT are available in Table 11a-d.

Table 11a: Auditory Startle Test on Historical Controls for P20 Males. Values are depicted as mean ± standard deviation. Data

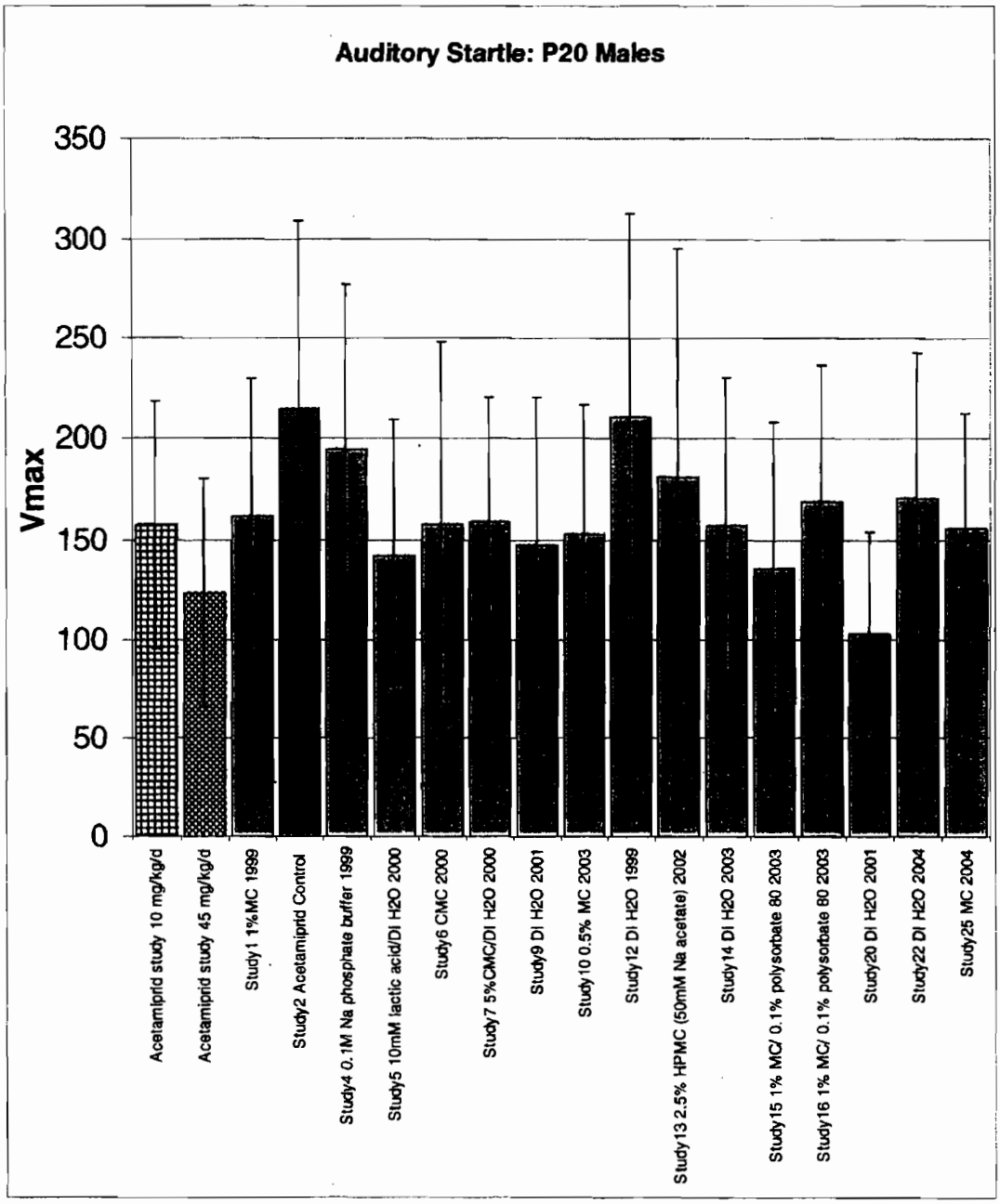


Table 11b: Auditory Startle Test on Historical Controls for P60 Males. Values are depicted as mean ± standard deviation

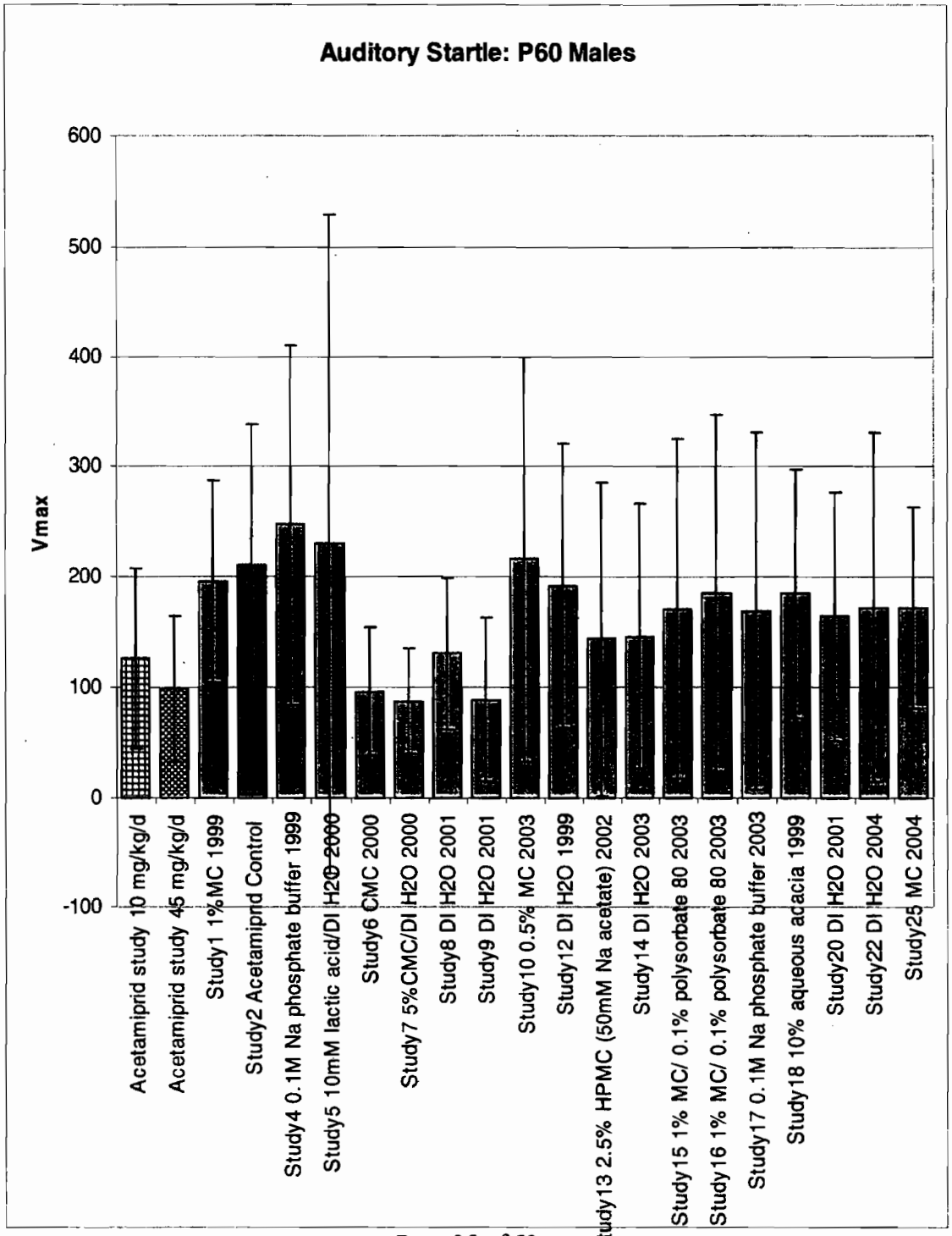


Table 11c: Auditory Startle Test on Historical Controls for P20 Females. Values are depicted as mean ± standard deviation

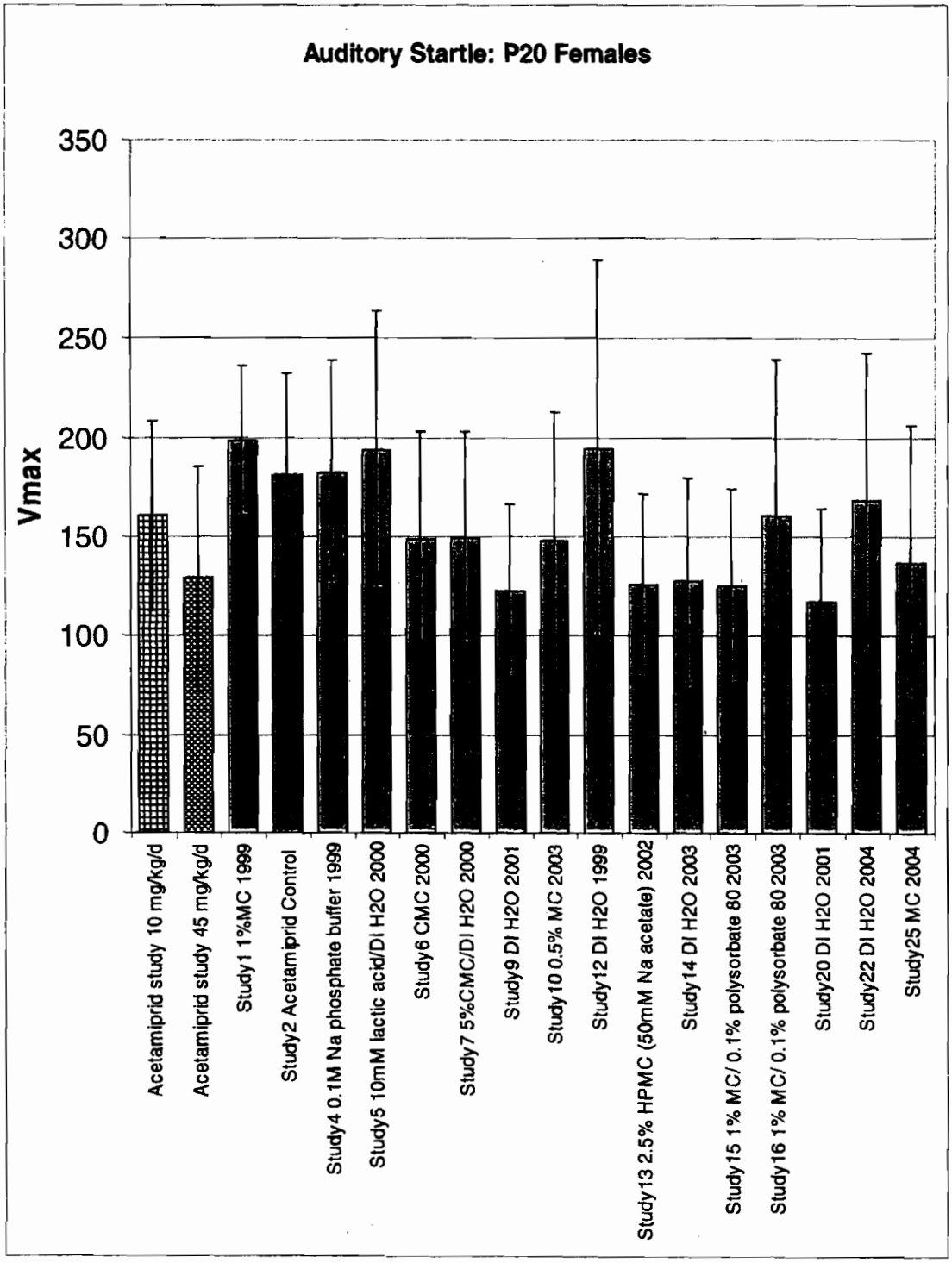
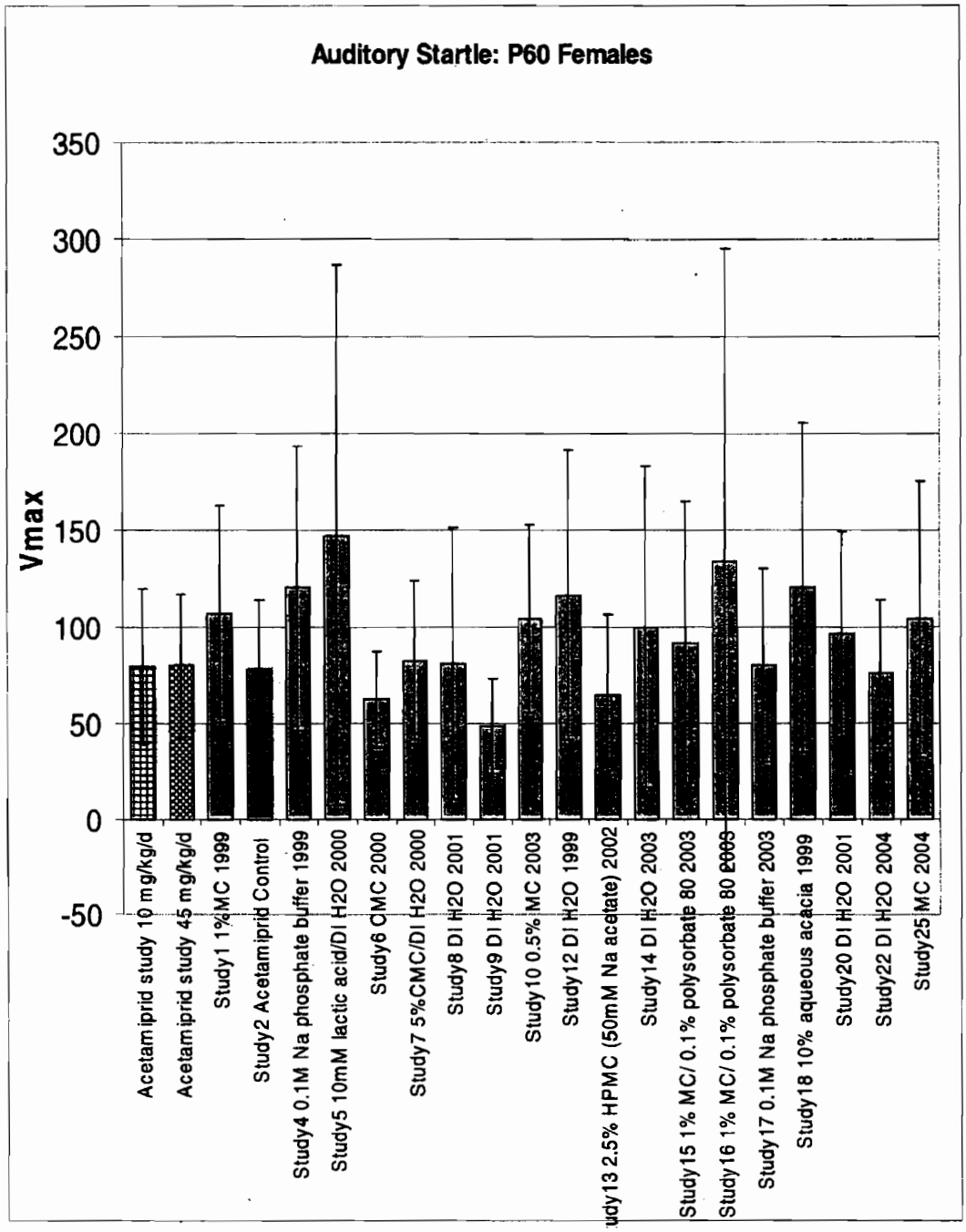


Table 11d: Auditory Startle Test on Historical Controls for P60 Females. Values are depicted as mean ± standard deviation



d. **Learning and memory testing:** The learning and memory data are presented in Tables 12 (PND 22) and 13 (PND 62). There were increases in trials 5-10 in path B in high-dose PND 22 males, but there was high variability in the data. No similar trend was seen in females. No difference in latency was seen in path A. Therefore, no conclusions may be drawn from these data.

TABLE 12. Water maze performance (mean ± SD) in offspring on PND 22 [n]

Day/Trial	Dose (mg/kg/day) 0	2.5	10	45
	Males			
Day 1 - Swimming Ability Mean Time (secs)	9.05±2.76	9.34±3.28	9.14±1.82	9.61±3.09
Trial 1 (Day 2) - Path A Mean Time (secs) Mean No. Errors	73.33±58.61 13±11.7	130.15±51.04 23±7.2	58.52±28.78 11±6.0	84.27±40.83 18±11.1
Trial 2 (Day2) - Path A Mean Time (secs) Mean No. Errors	73.23±50.26 13±10.1	103.72±40.49 20±9.0	67.17±36.92 13±7.5	87.32±53.63 19±2.9
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	64.44±50.18 13±13.4	43.72±19.43 10±5.6	44.79±27.78 9±6.9	65.55±56.52 16±15.5
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	55.04±44.08 9±9.4	39.44±28.10 8±8.8	49.78±30.73 10±7.5	70.52±47.38 18±16.8
Trial 5 (Day 4) - Path B Mean Time (secs) Mean No. Errors	136.42±63.74 26±13.2	151.99±50.77 25±11.5	133.23±67.78 24±13.0	156.28±50.12 29±9.8
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	103.56±59.38 19±13.5	130.14±65.70 21±12.6	122.87±66.46 23±12.5	116.08±72.48 21±12.8
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	108.32±67.51 19±14.0	113.39±63.61 19±12.5	106.42±53.61 20±7.90	131.88±58.77 25±11.7
Trial 8 (Day 5) - Path B Mean Time (secs) Mean No. Errors	59.91±58.36 9±9.7	79.82±62.11 14±12.4	62.37±55.00 14±17.1	101.54±55.20 19±11.1
Trial 9 (Day 6) - Path B Mean Time (secs) Mean No. Errors	69.33±64.76 12±14.9	69.80±69.93 13±14.2	73.41±64.64 17±18.0	96.22±74.67 18±13.4
Trial 10 (Day 6) - Path B Mean Time (secs) Mean No. Errors	40.62±52.20 7±10.0	43.43±42.24 7±7.5	55.66±48.44 11±13.6	52.80±48.48 8±8.0
Trail 11 (Day 7) - Path A (Probe) Mean Time (secs) Mean No. Errors	80.73±46.75 22±14.1	52.81±16.35 15±6.0	74.19±46.62 21±12.9	76.05±53.20 20±16.3
Trial 12 (Day 7) - Path A (Probe)				

TABLE 12. Water maze performance (mean ± SD) in offspring on PND 22 [n]				
	Dose (mg/kg/day)			
Day/Trial	0	2.5	10	45
Mean Time (secs) Mean No. Errors	46.80±36.28 10±8.5	46.11±21.73 10±6.4	57.00±30.17 14±9.6	46.64±21.13 11±6.8
Overall Biel (Trials 1-10) Mean Time (secs) Mean No. Errors	78.64±26.04 14±4.2	90.56±23.04 16±3.9	77.42±13.59 15±3.9	96.24±27.19 19±14.1
Overall Probe (Trials 11-12) Mean Time (secs) Mean No. Errors	63.76±31.46 16±9.2	49.46±12.94 13±3.9	65.58±24.17 17±6.5	61.35±30.13 15±9.4
Females				
Day 1 - Swimming Ability Mean Time (secs)	9.37±3.68	10.52±3.04	9.68±2.31	8.66±2.62
Trial 1 (Day 2) - Path A Mean Time (secs) Mean No. Errors	55.53±42.62 11±10.8	78.86±56.34 14±13.0	63.64±19.44 13±5.3	68.00±42.73 14±8.7
Trial 2 (Day2) - Path A Mean Time (secs) Mean No. Errors	69.45±59.16 14±13.2	57.64±36.95 12±9.6	81.75±46.13 16±10.7	110.68±66.31 22±13.9
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	72.27±54.32 15±12.7	62.69±44.46 16±13.1	60.51±55.01 14±15.3	50.13±24.95 12±7.7
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	38.68±21.46 6±5.2	62.82±47.33 15±14.5	48.73±38.54 10±9.3	45.66±41.56 10±10.8
Trial 5 (Day 4) - Path B Mean Time (secs) Mean No. Errors	147.68±53.57 23±10.8	162.75±36.40 32±8.8	120.28±64.37 19±11.1	173.99±19.02 30±6.4
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	147.50±53.87 22±11.3	142.70±59.93 31±15.8	108.11±64.04 22±14.9	98.03±63.11 18±11.0
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	101.29±51.62 16±7.2	93.50±58.96 17±11.0	132.46±54.35 26±11.1	114.96±63.57 22±11.8
Trial 8 (Day 5) - Path B Mean Time (secs) Mean No. Errors	61.08±63.53 9±13.2	92.52±75.93 17±14.2	77.35±55.09 14±15.4	74.22±62.13 14±11.7
Trial 9 (Day 6) - Path B Mean Time (secs) Mean No. Errors	99.92±70.16 17±14.0	67.36±66.20 13±14.3	68.34±58.25 15±13.4	86.25±60.94 18±13.7
Trial 10 (Day 6) - Path B Mean Time (secs) Mean No. Errors	61.78±45.75 12±9.2	59.54±54.89 12±14.0	41.25±43.73 8±10.5	32.13±19.27 5±4.7
Trail 11 (Day 7) - Path A (Probe) Mean Time (secs) Mean No. Errors	92.07±67.58 24±19.4	86.24±58.76 23±17.2	78.28±58.23 24±19.8	67.57±37.68 21±13.4
Trial 12 (Day 7) - Path A (Probe)				

TABLE 12. Water maze performance (mean ± SD) in offspring on PND 22 [a]				
Day/Trial	Dose (mg/kg/day)			
	0	2.5	10	45
Mean Time (secs) Mean No. Errors	73.81±36.97 18±10.6	44.47±22.29 11±7.5	45.12±31.14 10±9.4	54.06±29.97 13±7.5
Overall Biel (Trials 1-10) Mean Time (secs) Mean No. Errors	 85.52±21.69 15±4.1	 88.04±22.88 18±6.1	 80.24±19.15 16±5.1	 85.40±24.18 16±4.6
Overall Probe (Trials 11-12) Mean Time (secs) Mean No. Errors	 82.94±48.91 21±14.0	 65.35±34.02 17±10.2	 61.70±36.85 17±11.9	 60.81±22.67 17±7.2

[a] Data obtained from pages 210-217, MRID 46255619.
A = Forward route through maze
B = Reverse route through maze
N=10

TABLE 13. Water maze performance (mean ± SD) in offspring on PND 62 [a]				
Day/Trial	Dose (mg/kg/day)			
	0	2.5	10	45
	Males			
Day 1 - Swimming Ability Mean Time (secs)	7.15±2.92	6.39±1.42	6.67±2.05	6.81±2.03
Trial 1 (Day 2) - Path A Mean Time (secs) Mean No. Errors	 76.15±48.17 15±10.4	 81.07±61.98 18±16.0	 74.21±41.28 15±5.3	 82.95±58.23 20±13.4
Trial 2 (Day2) - Path A Mean Time (secs) Mean No. Errors	 35.11±17.01 8±5.7	 47.84±49.06 12±14.9	 58.08±50.89 13±12.0	 65.64±53.72 14±13.0
Trial 3 (Day 3) - Path A Mean Time (secs) Mean No. Errors	 66.01±74.93 14±16.8	 59.69±56.73 14±15.2	 46.66±36.70 11±9.4	 52.01±51.23 15±15.8
Trial 4 (Day 3) - Path A Mean Time (secs) Mean No. Errors	 22.03±16.55 4±3.6	 24.45±24.93 5±6.9	 21.89±12.83 5±4.2	 38.46±37.41 10±10.7
Trial 5 (Day 4) - Path B Mean Time (secs) Mean No. Errors	 164.40±33.41 32±10.7	 133.39±63.51 25±14.7	 120.05±78.24 24±16.5	 101.91±69.55 21±17.1
Trial 6 (Day 4) - Path B Mean Time (secs) Mean No. Errors	 109.02±61.10 18±8.6	 98.92±63.57 19±14.3	 76.93±53.70 13±10.0	 109.74±61.92 22±13.6
Trial 7 (Day 5) - Path B Mean Time (secs) Mean No. Errors	 47.77±51.42 8±9.1	 61.58±65.57 10±11.6	 65.91±53.91 14±11.5	 62.22±55.22 12±13.8

TABLE 13. Water maze performance (mean ± SD) in offspring on PND 62 [a]

Day/Trial	Dose (mg/kg/day) 0	2.5	10	45
Trial 8 (Day 5) - Path B				
Mean Time (secs)	39.01±52.29	36.26±41.77	45.49±55.33	17.79±11.62
Mean No. Errors	6±6.2	7±9.8	8±8.6	3±5.2
Trial 9 (Day 6) - Path B				
Mean Time (secs)	47.67±50.25	35.95±23.47	60.32±66.21	28.76±18.51
Mean No. Errors	6±3.7	7±6.6	14±16.2	5±4.6
Trial 10 (Day 6) - Path B				
Mean Time (secs)	34.88±49.54	20.76±9.00	36.77±44.28	21.57±19.45
Mean No. Errors	4±4.3	3±2.2	6±7.7	4±6.9
Trail 11 (Day 7) - Path A (Probe)				
Mean Time (secs)	48.50±17.95	81.18±51.07	67.76±34.78	81.34±62.29
Mean No. Errors	12±7.8	18±12.4	17±10.7	21±18.0
Trial 12 (Day 7) - Path A (Probe)				
Mean Time (secs)	48.56±36.14	37.93±45.63	36.30±16.16	50.95±28.58
Mean No. Errors	11±10.5	7±11.8	8±6.6	12±7.8
Overall Biel (Trials 1-10)				
Mean Time (secs)	64.20±27.62	59.99±17.38	60.63±21.68	58.10±14.54
Mean No. Errors	11±2.3	12±4.4	12±4.0	12±4.3
Overall Probe (Trials 11-12)				
Mean Time (secs)	48.53±23.05	59.55±30.25	52.03±20.84	66.15±40.85
Mean No. Errors	12±7.8	13±7.4	13±7.3	16±10.4
Females				
Day 1 - Swimming Ability				
Mean Time (secs)	7.66±1.25	6.68±1.15	7.44±1.60	6.65±1.74
Trial 1 (Day 2) - Path A				
Mean Time (secs)	45.34±31.29	66.04±24.86	73.32±44.71	64.28±62.71
Mean No. Errors	9±7.9	15±7.3	15±10.7	12±11.3
Trial 2 (Day2) - Path A				
Mean Time (secs)	51.56±40.67	36.94±18.94	37.66±21.67	39.62±30.68
Mean No. Errors	11±11.1	7±4.7	6±5.0	8±7.1
Trial 3 (Day 3) - Path A				
Mean Time (secs)	43.79±28.27	55.52±39.86	43.40±21.80	41.11±29.23
Mean No. Errors	9±6.1	14±11.0	10±7.8	9±7.2
Trial 4 (Day 3) - Path A				
Mean Time (secs)	28.69±13.46	38.74±24.15	26.32±12.90	30.58±17.70
Mean No. Errors	5±5.1	11±8.7	5±3.8	7±4.4
Trial 5 (Day 4) - Path B				
Mean Time (secs)	162.37±36.04	114.24±60.87	136.16±59.71	106.90±66.42
Mean No. Errors	31±8.2	23±12.8	31±14.6	19±12.0
Trial 6 (Day 4) - Path B				
Mean Time (secs)	92.01±57.63	108.64±68.06	80.32±60.49	96.57±59.84
Mean No. Errors	19±14.1	21±16.6	17±13.1	17±11.9
Trial 7 (Day 5) - Path B				
Mean Time (secs)	64.38 ±66.22	91.22±66.70	52.07±49.64	58.18±50.28
Mean No. Errors	9±10.9	17±14.8	9±10.4	12±11.4

TABLE 13. Water maze performance (mean ± SD) in offspring on PND 62 [a]

Day/Trial	Dose (mg/kg/day) 0	2.5	10	45
Trial 8 (Day 5) - Path B				
Mean Time (secs)	68.04±70.27	56.06±58.56	58.77±62.22	56.98±58.02
Mean No. Errors	12±14.4	11±13.0	11±13.0	9±8.9
Trial 9 (Day 6) - Path B				
Mean Time (secs)	54.04±57.98	36.12±32.54	57.97±51.38	53.40±55.33
Mean No. Errors	9±11.8	7±9.6	10±8.3	11±14.8
Trial 10 (Day 6) - Path B				
Mean Time (secs)	36.19±53.30	19.86±12.52	55.08±67.42	31.12±22.28
Mean No. Errors	5±10.4	2±3.9	10±13.5	4±4.2
Trail 11 (Day 7) - Path A (Probe)				
Mean Time (secs)	76.58±59.50	62.01±35.98	59.81±21.89	79.16±51.36
Mean No. Errors	16±14.2	18±9.6	14±4.8	18±11.5
Trial 12 (Day 7) - Path A (Probe)				
Mean Time (secs)	46.04±22.49	38.84±16.81	42.20±16.36	37.53±27.65
Mean No. Errors	8±5.1	8±5.1	8±3.6	7±6.8
Overall Biel (Trials 1-10)				
Mean Time (secs)	64.64±18.99	62.34±14.75	62.11±28.86	57.87±24.46
Mean No. Errors	12±3.4	13±4.2	12±4.5	11±4.2
Overall Probe (Trials 11-12)				
Mean Time (secs)	61.31±28.88	50.43±22.07	51.01±16.32	58.35±34.30
Mean No. Errors	12±7.3	13±5.8	11±3.8	12±7.8

[a] Data obtained from pages 210-225, MRID 46255619.
A = Forward route through maze
B = Reverse route through maze
N=10

5. Postmortem results:

a. **Brain weight, width and length:** No treatment-related effects were noted on brain weight or length on PNDs 11 and 72. Brain width was significantly decreased (95% of control value) in males at 45 mg/kg/day on PND72. Mean brain weight, length and width data are presented in Table 14.

b. **Macroscopic examination:** An increased number of pups at 45 mg/kg/day which were found dead or euthanized *in extremis* did not have milk present in the stomach at necropsy. One pup at 45 mg/kg/day also had a malrotated hindlimb and a filamentous tail. In offspring from litters that either failed to meet the sex ratio criteria or were not selected for behavioral evaluation, one pup in the 10 mg/kg/day group had dilated renal pelves. No treatment-related findings were observed in offspring euthanized on PND 72 (not selected for neuropathology) or in pups selected for brain weight on PNDs 11 and 72.

TABLE 14. Mean (±SD) Brain Weight Data in Offspring [a]				
Parameter	Dose (mg/kg/day)			
	0	2.5	10	45
Males				
Day 11				
Terminal body weight (g)	24±2.4	24±3.9	25±2.0	24±3.0
Brain weight (g)	1.32±0.12	1.29±0.15	1.32±0.10	1.25±0.10
Brain length (mm)	16.1±0.83	16.1±0.82	16.2±0.53	16.0±0.68
Brain width (mm)	13.6±0.45	13.5±0.64	13.6±0.49	13.4±0.44
Brain-to-body weight ratio (gms/100 gms)	5.53±0.41	5.52±0.61	5.35±0.38	5.34±0.49
Day 72				
Terminal body weight (g)	434±39.7	433±26.8	433±25.8	401±31.4
Brain weight (g)	1.95±0.06	2.00±0.09	1.97±0.08	1.90±0.07
Brain length (mm)	20.6±0.94	20.9±0.55	20.5±0.49	20.2±0.71
Brain width (mm)	15.3±0.45	15.2±0.41	15.1±0.25	14.6**±0.65 (95)
Brain-to-body weight ratio (gms/100 gms)	0.45±0.03	0.46±0.03	0.46±0.02	0.48±0.03
Females				
Day 11				
Terminal body weight (g)	22±3.2	23±3.2	25*±2.3	22±2.7
Brain weight (g)	1.27±0.15	1.26±0.13	1.30±0.10	1.23±0.12
Brain length (mm)	15.9±0.70	15.9±0.36	16.0±0.51	15.8±0.48
Brain width (mm)	13.3±0.61	13.5±0.57	13.7±0.60	13.3±0.65
Brain-to-body weight ratio (gms/100 gms)	5.70±0.40	5.62±0.56	5.34±0.44	5.63±0.55
Day 72				
Terminal body weight (g)	256±23.8	254±24.8	255±20.0	239±16.8
Brain weight (g)	1.78±0.07	1.82±0.10	1.76±0.06	1.75±0.09
Brain length (mm)	19.8±0.86	19.9±0.81	19.8±0.71	19.5±1.18
Brain width (mm)	14.8±0.49	14.8±0.59	14.8±0.56	14.1±1.04
Brain-to-body weight ratio (gms/100 gms)	0.70±0.08	0.72±0.06	0.69±0.04	0.74±0.06

[a] Data obtained from pages 231-238, MRID 46255619
N=21-22
* Statistically significantly different from control value, $p<0.05$

c. **Neuropathology:**

1) **Microscopic examination:** No treatment-related effects were reported in male and female offspring on PNDs 11 and 72. On PND 72, one control male had focal degeneration of the lumbar spinal cord characterized by axonal swelling within the cord. Minimal to mild degeneration of several nerves, including the sciatic, sural, peroneal, tibial and lumbar dorsal and ventral root fibers were observed in both the control and 45 mg/kg/day groups with no increased incidence in the treated group. The degeneration was characterized by swelling, distension and/or vacuolation of the myelin sheath with eosinophilic debris within the distended area.

2) **Brain Morphometry:** Morphometric evaluation data are presented in Table 15. On PND 11, the vertical height between hippocampal pyramidal neurons (Level 3) in females at 45 mg/kg/day was significantly increased. On PND 72, the length of the ventral limb of the dentate hilus in the hippocampal formation (Level 3) of females at 45 mg/kg/day was significantly decreased.

TABLE 15. Mean (cm±SD) brain morphometric data [a]

Parameter	Dose (mg/kg/day)			
	Males		Females	
	0	45	0	45
Day 11				
Level 1				
Ht Hemisphere	0.83±0.06	0.84±0.08	0.83±0.08	0.84±0.04
V Thickness cortex	0.25±0.04	0.25±0.02	0.23±0.03	0.26±0.03
Level 3				
Radial thickness cortex	0.16±0.01	0.17±0.01	0.15±0.02	0.16±0.15
V Ht BTW Hippocampal Pyramidal Neuron Layers	0.11±0.01	0.11±0.01	0.10±0.01	0.11*±0.01
V Ht Dentate Hilus	0.06±0.00	0.05±0.00	0.05±0.01	0.05±0.01
Length ventral limb dentate hilus	0.12±0.01	0.12±0.01	0.12±0.01	0.12±0.02
Level 5				
V Thickness of pons	0.30±0.04	0.33±0.01	0.32±0.04	0.32±0.03
Base of lobule 9	0.06±0.00	0.06±0.01	0.06±0.00	0.06±0.01
Day 72				
Level 1				
Ht Hemisphere	0.67±0.06	0.67±0.02	0.67±0.05	0.66±0.05
V Thickness cortex	0.18±0.02	0.19±0.02	0.17±0.01	0.17±0.02
Level 3				
Radial thickness cortex	0.13±0.01	0.13±0.01	0.13±0.02	0.12±0.01
V Ht BTW Hippocampal Pyramidal Neuron Layers	0.10±0.01	0.10±0.01	0.09±0.01	0.09±0.00
V Ht Dentate Hilus	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00
Length ventral limb dentate hilus	0.14±0.02	0.12±0.01	0.13±0.01	0.11*±0.01
Level 5				
V Thickness of pons	0.33±0.02	0.33±0.02	0.31±0.03	0.31±0.02
Base of lobule 9	0.07±0.00	0.06±0.01	0.06±0.01	0.06±0.01

a Data obtained from pages 243-246 and 259-262, MRID 46255619.
N = 8-10
* Significantly different from control, $p<0.051$

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that evidence of maternal toxicity at 45 mg/kg/day included a single mortality and reductions in body weight gain and food consumption. Evidence of offspring toxicity at 45 mg/kg/day included early postnatal mortality and decreased post-weaning body weight. The No Observed Adverse Effect Level (NOAEL) for maternal toxicity, developmental toxicity and developmental neurotoxicity was 10 mg/kg/day.

B. **REVIEWER COMMENTS:** One dam in the 45 mg/kg/day group died during parturition. There were no clinical signs of toxicity prior to death and no findings on gross necropsy. The incidences of certain clinical signs (hair loss and dried red material on the forelimbs, scabbing on the forelimbs and red material around the nose) were increased in the treated groups. Salivation prior to dosing was increased in females at 45 mg/kg/day. All animals in the 45 mg/kg/day group and nine animals at 10 mg/kg/day were observed to wipe their mouths on the cage bedding following dosing. The significance of these findings is unclear, although they may explain the transient body weight loss in the high dose group during gestation (see below).

No treated-related findings were observed during the FOB observations on GDs 6 and 12 and LDs 4 and 7.

Mean body weight was slightly but significantly decreased (4-5%) on GDs 9-20 in females at 45 mg/kg/day. A mean body weight loss was observed in dams at 45 mg/kg/day during GDs 6-9. For the entire gestation period (GDs 6-20), mean body weight gain was significantly decreased in females at 45 mg/kg/day. On LD 1, mean body weight was significantly decreased in females at 45 mg/kg/day but was comparable to controls during the remainder of lactation. Mean food consumption in females at 45 mg/kg/day was significantly decreased during GDs 6-9 and 9-12 and for the entire gestation period (GDs 6-20). The body weight loss experienced by high dose dams during gestation may be related to low food consumption because of an inhibited desire to eat following dosing (see clinical signs above) rather than a treatment-related effect due to toxicity. Importantly, the body weights recovered during lactation even though gavage dosing continued through the lactation period (see pages 14-15 for more on a response to a registrant rebuttal on this issue).

Live litter size (PND 0) and postnatal survival on PNDs 0-1 and 0-4 were decreased at 45 mg/kg/day. Three females at 45 mg/kg/day had total litter loss on PND 1. Postnatal survival in the treated groups was similar to controls for the remainder of the pre-weaning period. The total number of pups found dead, euthanized *in extremis* or due to death of the dam, and missing and presumed cannibalized was increased at 45 mg/kg/day during the pre-weaning period. All pups survived to their respective scheduled necropsies during the post-weaning period. The percentage of males on the day of birth was non-significantly decreased in the 45 mg/kg/day group; the finding is not considered treatment-related as there was no dose-response effect and the percentage was within the historical control range.

Mean body weight in female offspring at 45 mg/kg/day was slightly but significantly decreased on PND 1. Mean body weight gain in the treated groups was comparable to the controls during the pre-weaning period. Mean body weight and body weight gain were significantly decreased post-weaning in male and female offspring at 45 mg/kg/day. Mean body weight gain was also significantly decreased in males at 2.5 mg/kg/day on PNDs 42-49, 49-56 and 70-72 and 10 mg/kg/day on PNDs 70-72. These decreases are not considered treatment-related since there was no dose response. The mean age of preputial separation in males and vaginal opening in females was comparable to the control value. There were no treatment-related effects on FOB measurements.

During the auditory startle response testing, the maximum response amplitudes (V_{MAX}) and average response amplitudes (V_{AVE}) were decreased in treated males in a dose-responsive manner on PNDs 20 and 60. On PND 20, V_{MAX} and V_{AVE} were non-significantly reduced in females at 10 mg/kg/day and 45 mg/kg/day. It was noted that the values in control males (PND 20) were not increased compared to the historical control values of other DNT studies conducted in 1999. Response values for the other treated groups were within the historical range. Nevertheless, for males, maximum auditory startle response amplitude was decreased 27% (PND 20) and 40% (PND 60) at 10 mg/kg/day, and it was decreased 42% (PND 20) and 53% (PND 60) at 45 mg/kg/day. Decreases observed at 2.5 mg/kg/day on PND 20 (15%) and PND 60 (10%) were not considered to be toxicologically significant (see pp. 22-23 for additional information pertaining to a registrant rebuttal on this issue).

In the learning and memory assessment test, there were increases in trials 5-10 in path B in high-dose PND 22 males, but there was high variability in the data. No similar trend was seen in females. No difference in latency was seen in path A. Therefore, no conclusions may be drawn from these data.

No treatment-related effects were noted on brain weight and length on PNDs 11 and 72. Brain width was significantly decreased in males at 45 mg/kg/day on PND 72. Findings on macroscopic and microscopic examinations were in both control and treated animals with no increased incidence in treated groups. On morphometric examination of females at 45 mg/kg/day, the vertical height between hippocampal pyramidal neurons (Level 3) was significantly increased on PND 11 and the length of the ventral limb of the dentate hilus in the hippocampal formation (Level 3) was significantly decreased on PND 72. These findings are not considered treatment-related.

The maternal NOAEL is 10 mg/kg/day. The maternal LOAEL is 45 mg/kg/day based on a 4-5% decrease in body weight in dams during gestation.

The offspring LOAEL is 10 mg/kg/day based on a decreased maximum auditory startle response in males on PND 20 and PND 60. The offspring NOAEL is 2.5 mg/kg/day.

This study is classified **Acceptable/Non guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, ' 83-6); OECD 426 (draft). This study is

classified as non-guideline due to the deficiencies listed below and the pending review of the submitted positive control data.

C. STUDY DEFICIENCIES:.

Inadequate assessment of motor activity and learning and memory

APPENDIX I

ACETAMIPRID DNT STUDY (WIL-21193): Statistical Analyses for Auditory Startle Response. DP Barcode D341525
MEMO: July 24, 2007 from James Nguyen

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

July 24, 2007

MEMORANDUM

SUBJECT: ACETAMIPRID DNT STUDY (WIL-21193): Statistical Analyses for Auditory Startle Response.
DP Barcode D341525
PC Code 099050

FROM: James Nguyen, Mathematical Statistician
Chemistry and Exposure Branch
Health Effects Division (7509P)

THRU: David J. Miller, Chief
Chemistry and Exposure Branch
Health Effects Division (7509P)

TO: Jess Rowland, Co-Chair
Louis Scarano, Co-Chair
DNT Workgroup
Health Effects Division (7509P)

and

Thomas Moriarty, Risk Assessor
Kimberly Harper, Toxicologist
Registration Action Branch 2
Health Effects Division(7509P0

I. Overview

The Health Effects Division (HED) reviewed a developmental neurotoxicity (DNT) study of rats exposed to acetamiprid (MRID No. 46255619; TXR No. 0052563) which was sponsored by Nippon Soda Co. Ltd. and conducted by WIL Research Laboratories, LLC. The HED review concluded that there was no maternal LOAEL (NOAEL at high dose of 45 mg/kg/day) and the offspring LOAEL was the mid dose of 10 mg/kg/day (the offspring NOAEL was the low dose of 2.5 mg/kg/day) based on a decreased maximum auditory startle response in males on post-natal day (PND) 20 and PND 60. The registrant disagreed with these conclusions and submitted a rebuttal (MRID 46779201) to the DNT Data Evaluation Record (DER), including

a statistical analysis of maximum auditory startle response in males and females on PND 20 and PND 60 performed by BioSTAT Consultants, Inc. This analysis showed that there were no statistically significant effects at the mid dose (10 mg/kg/day) level for males or females. However, the analysis did indicate that there was a marginally statistically significant trend in maximum auditory startle response going from the control to low to medium dose groups for males at PND 60, but no other significant trends for the other sex and PND combinations.

Chemistry and Exposure Branch (CEB) was requested to duplicate the registrant's analysis for confirmatory purposes, regardless of whether the analysis was considered to be the most appropriate given the structure of the data from the DNT study. CEB was able to reproduce most of the results from the BioSTAT analysis (submitted as part of Nippon Soda rebuttal MRID No. 46779201) and agreed with the conclusions drawn from the results of the registrant-submitted rebuttal. However, the DNT Workgroup determined that the effect at the mid dose was biologically significant and treatment related. Details and further discussion of the DNT Workgroup rationale are provided in the DER for the DNT study (TXR# 0052563). Following the release of the DNT Workgroup's conclusion, the registrant submitted a new analysis performed by Exponent, Inc. (MRID 47181101). Rather than just confirming the results of the new analysis, CEB was requested to provide a critical review of the registrant-submitted analysis and provide support by means of a statistical analysis of the maximum auditory startle response data. In general, CEB had concerns with the incorrect reporting of the results of some of the significance tests, as well as the selected model used to analyze the data which did not allow the statistical power of the DNT study design to be optimized. Additionally, CEB provided a statistical analysis which utilized a more appropriate model given the structure of the data and also increased the power of the statistical tests to detect significant differences and trends. Additional details of the statistical analyses performed by BioSTAT, Exponent, and CEB are provided in the next section. A summary of the data is provided in Attachment 1. The relevant SAS code and outputs are provided in Attachment 2.

II. Detailed Analysis

A. BioSTAT Consultants, Inc. Analysis

In the registrant's original rebuttal, BioSTAT Consultants, Inc. used the Proc Mixed procedure in SAS® to perform a multilevel analysis which incorporated random effects to model the repeated measures design of the DNT study. The covariance structure across time was first-order autoregressive [AR(1)] with heterogeneity in the covariance structure for different levels of treatment (group = dosegp). In this submission, there were four separate analyses for four subsets of maximum auditory startle response data: PND 20 males, PND 60 males, PND 20 females, and PND 60 females. For each of the four separate analyses, Dunnett's test was used to determine if there were significant differences between the group means of the treatment groups compared to that of the control group mean. The Tukey linear sequential trend test was also used to determine if there were any significant trends in maximum auditory startle response for the following dose group sequences: control to low (C-L); control to low to mid (C-L-M); and control to low to mid to high (C-L-M-H).

CEB was able to reproduce most of the results of the BioSTAT analysis. The results of this confirmatory analysis are provided in Table 1. The significantly different pairs of group means

(based on Dunnett's test) and significant treatment group response trends (based on Tukey sequential trend test) are bolded. The results of the statistical analysis showed a statistically significant difference between the means of the high dose groups and the control groups for the PND 20 males, and that there was significant trend in the response for the dose group sequence C-L-M-H for the PND 60 males, the PND 20 males, and PND 20 females. The mid dose and low dose group means were not statistically significantly different from the control means for any of the four separate analyses. Also, the C-L-M and C-L dose group trends were not significant for any of the four separate analyses.

CEB notes that the statistical power of detecting a significant difference in dose group means or a significant response trend is reduced by performing separate analyses for each sex and PND period. The BioSTAT analysis did not take advantage of other multilevel models that can combine male and female data from the PND 20 and PND 60 periods by including additional fixed effects resulting in a greater ability (i.e. higher power) to detect significant differences and trends. The DNT Workgroup determined that the response of the mid dose group (10 mg/kg/day) was biologically significant and is the offspring LOAEL, and the low dose (2.5 mg/kg/day) is the offspring NOAEL.

Table 1: Results of acetamiprid auditory startle data analysis performed by CEB (to confirm the BioSTAT analysis)

Subset	Dunnett's test		Tukey linear sequential trend test	
	Pairs	**p-value**	**Trend**	**p-value**
Male – PND20	**C – H**	**0.021**	**C – L – M – H**	**0.007**
	C – M	0.188	C – L – M	0.090
	C – L	0.670	C – L	--
Male – PND60	C – H	0.069	**C – L – M – H**	**0.018**
	C – M	0.192	C – L – M	0.054[a]
	C – L	0.920	C – L	--
Female – PND20	C – H	0.088	**C – L – M – H**	**0.022**
	C – M	0.651	C – L – M	0.267[a]
	C – L	1.000	C – L	--
Female – PND60	C – H	0.998	C – L – M – H	0.879
	C – M	0.999	C – L – M	--
	C – L	1.000	C – L	--

[a]The results of these significance tests differ from those of the BioSTAT analysis but do not alter the conclusions of the analysis.

B. Exponent, Inc. Analysis

Following the release of the DNT Workgroup's conclusion, the registrant submitted a new statistical analysis conducted by Exponent, Inc. (MRID 47181101) using the same data. In this submission, the registrant provided two additional analyses: combined males and females on PND 20, and combined males and females on PND 60. The results of Exponent analyses are shown in Table 2. The results from this new submission confirm the results from the registrant's previous submission. Specifically, no statistically significant effects were seen at

the mid dose. The registrant proposed the mid dose (10 mg/kg/day) as the NOAEL and the high dose (45 mg/kg/day) as the offspring LOAEL since there was no statistically significant difference found in analysis for the mid dose.

However, CEB has some statistical concerns with the Exponent analysis submitted by the registrant. The Tukey sequential linear trend test in the submitted analysis was not correct. The p-values of the trend test reported in the Exponent analysis were not associated with the Tukey linear sequential trend test. CEB has verified the analysis and found that these p-values were instead from the global F-test in ANOVA, which is used to determine if there is *at least one* group mean different from the other group means. Another problem, following from incorrectly using the F-test in ANOVA rather than the Tukey linear sequential trend test, was the incorrect elimination of the high dose data to produce separate subsets of data for testing the C-L-M sequence.

More importantly, CEB also believes that the multilevel model used in the later (Exponent) submission is not the most appropriate since it did not combine the two PND periods. The most appropriate multilevel model - which would have greater power to detect significant differences and trends - would combine the sexes and PND periods into a single analysis using fixed effects. The number of subjects (offspring) involved in this single analysis would be two and four times larger than the number of subjects used in the other analyses performed by Exponent. The larger number of subjects included in the CEB analysis should result in higher statistical power.

Table 2: Results of acetamiprid auditory startle data analysis performed by Exponent (Reproduced from Submission MRID 47181101)

		Repeated Measures Analysis of Variance					Post-hoc mean contrast comparisons		
		Sequential Dose Analysis		Gender	Dose x Trial	Dose x Gender		Tukey-Kramer	Dunnett
Period	Gender	Doses Used	Dose p-value	p-value	p-value	p-value	Test	p-value	p-value
20	M	C-L-M-H	**0.053**		0.436		C v H	**0.041**	**0.022**
		C-L-M	0.231				C v M	0.306	0.188
		C-L					C v L	0.811	0.670
20	F	C-L-M-H	0.123		0.354		C v H	0.142	0.088
		C-L-M					C v M	0.766	0.651
		C-L					C v L	1.000	1.000
20	M–F	C-L-M-H	**0.003**	0.653	0.355	0.772	C v H	**0.004**	**0.002**
		C-L-M	0.131	0.536			C v M	0.201	0.119
		C-L		0.433			C v L	0.889	0.793
60	M	C-L-M-H	**0.046**		0.787		C v H	0.138	0.069
		C-L-M	0.122				C v M	0.344	0.192
		C-L					C v L	0.975	0.920
60	F	C-L-M-H	0.999		0.937		C v H	0.999	0.998
		C-L-M					C v M	1.000	0.999
		C-L					C v L	1.000	1.000
60	M–F	C-L-M-H	**0.048**	**<0.001**	0.646	**0.038**	C v H	**0.149**	**0.076**
		C-L-M	0.150	**<0.001**		0.134	C v M	**0.382**	**0.218**
		C-L		**<0.001**			C v L	**0.979**	**0.935**

C. CEB Analysis

CEB used a mixed-effect model to perform an analysis of the combined data from males and females on PND 20 and PND 60. The analysis was performed using Proc Mixed in SAS® with a first-order autoregressive [AR(1)] correlation matrix for the errors within subject across time; heterogeneity in the covariance structure for the sex by period by dose term was specified [group = sex*period*dosegp]. A full model with all interaction terms was first explored before deriving a final best model (the SAS code for this model and accompanying output has been included in Attachment 2). Table 3 provides the results of Type 3 tests for the fixed effects of all variables including the interaction terms in the final model.

Table 3: Type 3 Tests of fixed effects in the acetamiprid auditory startle data analysis performed by CEB

Type 3 Tests of Fixed Effects				
Effect	**Num DF** [a]	**Den DF** [a]	**F Value** [a]	**Pr > F** [b]
SEX	1	110	15.71	0.0001
PERIOD	1	112	27.35	0.0001
DOSEGP	3	64.8	6.63	0.0006
TRIAL	4	429	29.59	0.0001
SEX*PERIOD	1	112	12.23	0.0007
SEX*DOSEGP	3	64.8	3.79	0.0143
SEX*TRIAL	4	429	1.44	0.2202
PERIOD*TRIAL	4	429	3.83	0.0045
SEX*PERIOD*TRIAL	4	429	3.24	0.0122

[a] Num DF and Den DF are numerator and denominator degrees of freedom. These are used with F value to compute the P-value in the F-test (F-distribution).
[b] Pr>F is the probability that F value larger than the critical F value. This is the P-value of the test.

Since the interaction SEX*DOSEGP was significant (p-value = 0.0143), the comparisons between dose group means were performed separately by sex. The results from the Dunnett's test and the Tukey sequential trend test are shown in Table 4. The p-values of the significant sequential trends have been bolded.

Table 4: Results of acetamiprid auditory startle data analysis performed by CEB

Type of Test		Trend or Pair	P-value
Tukey linear sequential trend test		**C-L-M-H**	**0.0001**
		C-L-M	**0.0045**
		C-L	0.4461
Dunnett's test	Male	**H – C**	**0.0015**
		M – C	**0.0321**
		L – C	0.7381
	Female	H – C	0.9344
		M – C	0.9963
		L – C	1.0000

The analysis performed by CEB indicates that for male rats, the mid dose and high dose group means were significantly different from the control group mean, but the low dose group mean was not significantly different from the control group mean. For female rats, there was no difference for any treatment groups compared to the control group. Also, the sequential trends C-L-M-H and C-L-M were found to be significant.

III. Conclusion

The CEB analysis using a more appropriate model for data structure and appropriate statistical methods indicated that for the maximum auditory startle response of male rats, the mid dose (10 mg/kg/day) and high dose (45 mg/kg/day) groups were significantly different from the control group (high dose for male: p-value = 0.0015, and mid dose for male: p-value = 0.0321), but the low dose group was not significantly different from the control. For female rats, there was no significant difference between the means of maximum auditory startle response of any of the treatment groups compared to the mean of the control group. Additionally, the Tukey sequential trend test indicated there were significant trends in the response for the dose group sequences C-L-M-H and C-L-M.

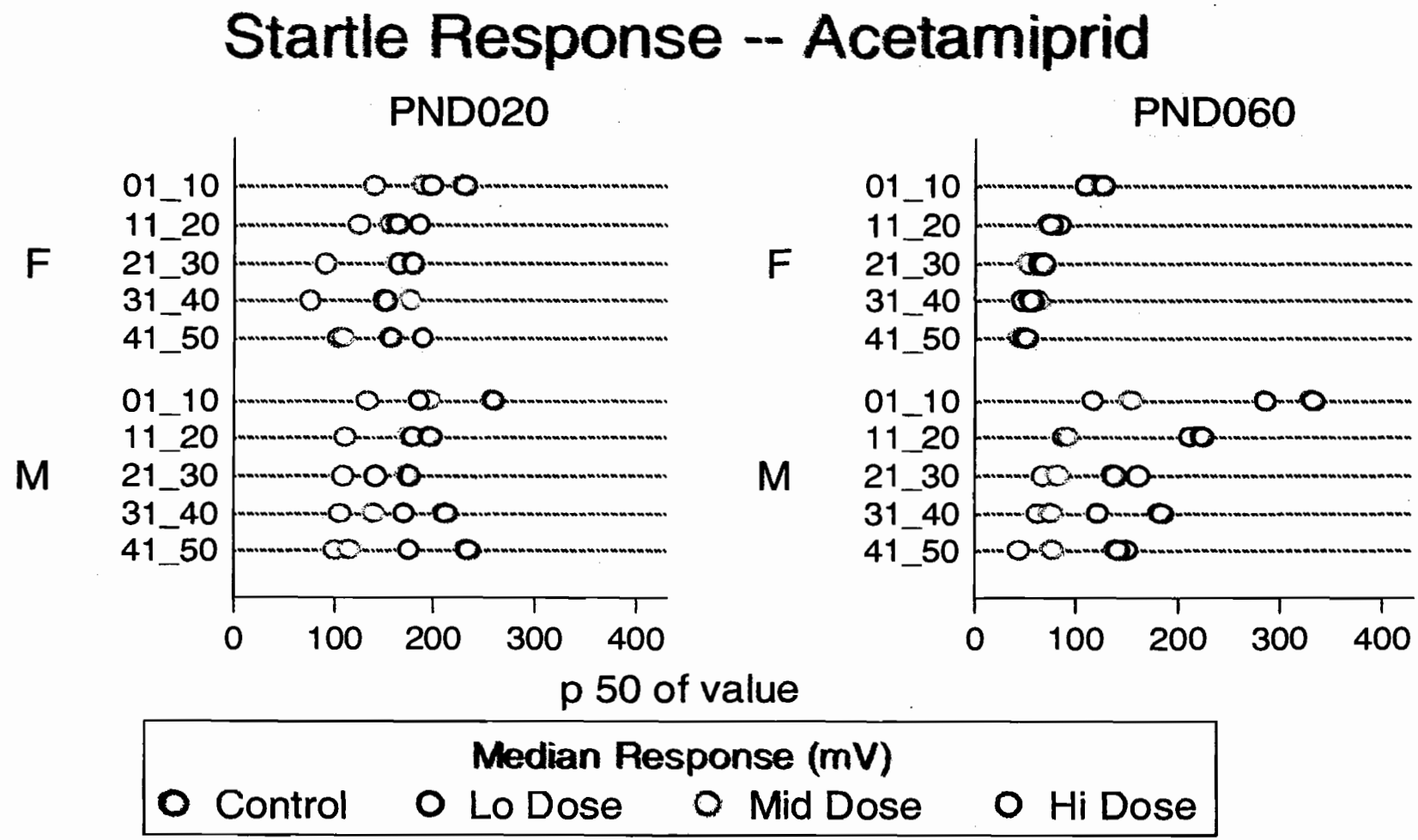
ATTACHMENT

ATTACHMENT 1
Summary Information for DNT Startle Response (Vmax)

period and dosegp	trial and sex														
	01_10			11_20			21_30			31_40			41_50		
	F	M	Total	F	M	Total	F	M	Total	F	M	Total	F	M	Total
PND020															
1	230.52	248.08	239.30	186.59	232.31	209.45	171.50	180.55	176.02	150.16	203.81	176.99	167.12	206.59	186.85
	59.61	99.60	80.40	65.19	136.66	106.82	56.97	81.29	68.48	63.45	106.66	89.74	61.18	113.87	91.24
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20
	227.50	257.80	239.40	161.90	193.50	173.15	177.10	173.05	177.10	149.15	209.00	168.30	154.10	231.75	173.25
2	199.28	204.22	201.75	164.94	201.32	183.13	169.52	156.64	163.08	182.07	176.48	179.27	196.59	174.68	185.63
	58.16	104.36	82.27	32.26	106.63	78.91	79.48	82.34	79.04	94.35	87.36	88.55	57.60	76.55	66.89
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20
	195.20	184.40	193.00	183.20	177.35	183.20	162.70	139.15	139.15	147.75	168.40	156.65	187.35	174.10	176.25
3	194.05	185.35	189.70	161.91	168.75	165.33	156.97	164.58	160.77	162.06	137.09	149.57	127.91	130.54	129.23
	48.49	69.27	58.37	67.51	72.29	68.17	70.42	88.03	77.69	52.63	66.32	59.66	62.44	58.43	58.87
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20
	187.85	194.45	187.85	154.95	172.85	167.35	161.85	171.50	171.50	175.00	138.55	156.10	106.60	113.95	113.25
4	168.26	142.92	155.59	133.44	117.73	125.58	137.38	120.65	129.01	103.26	117.40	110.33	102.68	118.28	110.48
	86.93	48.15	69.62	65.61	55.73	59.79	99.67	61.87	81.19	67.97	67.93	66.53	50.28	71.32	60.59
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20
	137.80	130.50	134.85	122.70	109.20	109.20	90.60	106.75	98.95	73.75	102.80	86.35	101.10	98.20	101.10
PND060															
1	116.75	329.67	223.21	81.31	237.49	159.40	72.27	175.86	124.07	61.75	176.00	118.88	58.75	130.43	94.59
	53.07	206.80	183.09	47.17	179.93	151.02	40.66	122.63	103.59	45.95	111.15	101.43	33.21	88.08	74.49
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20
	124.80	329.75	167.20	71.60	221.60	101.65	65.75	134.50	85.65	53.60	182.00	76.60	47.35	138.60	65.05
2	115.36	283.56	195.03	88.77	229.60	155.48	65.65	171.52	115.80	49.11	129.81	87.34	74.65	131.76	101.70
	42.37	122.11	122.35	42.55	123.51	113.60	25.02	97.09	86.33	25.89	76.53	68.21	59.15	83.78	75.68
	10	9	19	10	9	19	10	9	19	10	9	19	10	9	19
	107.80	283.00	165.90	80.75	210.80	132.80	61.65	159.50	83.40	44.95	119.30	62.80	48.30	146.50	70.70
3	124.39	204.93	164.66	87.56	139.57	113.57	57.85	102.10	79.97	64.60	104.23	84.42	63.36	79.36	71.36
	58.25	120.16	100.76	57.18	96.92	81.92	32.43	64.86	54.83	41.15	121.73	90.75	51.60	48.91	49.61
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20
	114.20	152.65	130.80	80.10	89.95	84.10	50.65	80.40	55.65	59.55	74.00	60.00	43.15	75.85	52.25
4	123.76	157.26	140.51	86.39	99.55	92.97	66.76	85.17	75.97	57.01	74.78	65.89	66.82	76.38	71.60
	42.64	119.22	88.82	66.05	70.68	66.92	40.98	58.16	49.87	37.82	51.53	44.93	52.86	90.78	72.47
	10	10	20	10	10	20	10	10	20	10	10	20	10	10	20

119.70	114.55	119.70	73.85	86.20	76.35	53.90	64.35	57.60	47.85	59.60	50.65	43.90	41.40	42.15

Values listed are: Mean, Standard deviation, Number of animals, and Median



Graphs by period

graph dot (median) value, over(dosegp) over(trial) over(sex) by(period, title("Startle Response -- Acetamiprid")) legend(rows(1) lab el (1 "Control") label (2 "Lo Dose") label (3 "Mid Dose") label (4 "Hi Dose") title("Median Response (mV)",size(medium))) scheme(vg_ outc)

ATTACHMENT 2

SAS CODE:

Data manipulation:

```
Data STARTLE.Vmax_combine;
      set startle;
      if type = "   VMAX";
      if period = "   PND020" then  trial1 =
      compress(trial||"_20");
      if period = "   PND060" then  trial1 =
      compress(trial||"_60");
      if sex = "   M" then SEX1 = "1";
      if sex = "   F" then SEX1 = "2";
run;

Data Vmax_combine;
      set STARTLE.Vmax_combine;
      if trial1 = "01_10_20" then trial2 = 1;
      if trial1 = "11_20_20" then trial2 = 2;
      if trial1 = "21_30_20" then trial2 = 3;
      if trial1 = "31_40_20" then trial2 = 4;
      if trial1 = "41_50_20" then trial2 = 5;
      if trial1 = "01_10_60" then trial2 = 6;
      if trial1 = "11_20_60" then trial2 = 7;
      if trial1 = "21_30_60" then trial2 = 8;
      if trial1 = "31_40_60" then trial2 = 9;
      if trial1 = "41_50_60" then trial2 = 10;
run;
```

Full Model with all variable and interaction terms:

```
Proc Mixed data = Vmax_combine noclprint noinfo method = ml;
      class sex period dosegp animlnum trial trial2;
      model value = sex period dosegp trial sex*period sex*dosegp
      sex*trial period*dosegp period*trial dosegp*trial
      sex*period*dosegp sex*dosegp*trial period*dosegp*trial
      sex*period*trial sex*period*dosegp*trial/ddfm = kr;
      repeated trial2/subject = animlnum type = ar(1) group =
      sex*period*dosegp;
run;
```

The output of this model (in output section) showed that there were some interaction terms having large p-values, which indicated that these interaction terms did not contribute much into the variation of the data. Removing these interaction terms should increase the available degree of freedom for the model. A chi-square test used to compare the fit of two models, full model and final best model, showed that the final best model below was a good model.

Final Best Model:

```
Proc Mixed data = Vmax_combine noclprint noinfo method = ml;
      class sex period dosegp animlnum trial trial2;
      model value = sex period dosegp trial sex*period sex*dosegp
      sex*trial period*trial sex*period*trial /ddfm = kr;
      repeated trial2/subject = animlnum type = ar(1) group =
      sex*period*dosegp;
      lsmeans sex*dosegp/pdiff adjust = dunnett;
      contrast 'C-L-M-H'  dosegp -3 -1 1 3;
      contrast 'C-L-M'  dosegp -2 -1 3 0;
      contrast 'C-L'  dosegp 1 -1 0 0;
run;

Proc Mixed data = Vmax_combine noclprint noinfo method = ml;
      class SEX1 period dosegp animlnum trial trial2;
      model value = SEX1 period dosegp trial SEX1*period
      SEX1*dosegp SEX1*trial period*trial SEX1*period*trial /ddfm
      = kr;
      repeated trial2/subject = animlnum type = ar(1) group =
      SEX1*period*dosegp;
      lsmeans SEX1*dosegp/pdiff adjust = dunnett;
run;
```

SAS OUTPUT:

Full Model with all variable and interaction terms:

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
SEX	1	86.5	17.91	<.0001
PERIOD	1	86.5	24.84	<.0001
DOSEGP	3	61.3	8.24	0.0001
TRIAL	4	436	31.08	<.0001
SEX*PERIOD	1	86.5	13.04	0.0005
SEX*DOSEGP	3	61.3	2.70	0.0536
SEX*TRIAL	4	436	2.44	0.0465
PERIOD*DOSEGP	3	61.3	0.25	0.8634
PERIOD*TRIAL	4	436	4.05	0.0031
DOSEGP*TRIAL	12	349	1.08	0.3718
SEX*PERIOD*DOSEGP	3	61.3	1.20	0.3189
SEX*DOSEGP*TRIAL	12	349	1.01	0.4350
PERIOD*DOSEGP*TRIAL	12	349	0.92	0.5249
SEX*PERIOD*TRIAL	4	436	3.56	0.0071
SEX*PERI*DOSEG*TRIAL	12	349	0.40	0.9622

Final Best Model:

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F

SEX	1	110	15.71	0.0001
PERIOD	1	112	27.35	<.0001
DOSEGP	3	64.8	6.63	0.0006
TRIAL	4	429	29.59	<.0001
SEX*PERIOD	1	112	12.23	0.0007
SEX*DOSEGP	3	64.8	3.79	0.0143
SEX*TRIAL	4	429	1.44	0.2202
PERIOD*TRIAL	4	429	3.83	0.0045
SEX*PERIOD*TRIAL	4	429	3.24	0.0122

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
C-L-M-H	1	54	18.27	<.0001
C-L-M	1	67.8	8.66	0.0045
C-L	1	51.9	0.59	0.4461

Differences of Least Squares Means

Effect	SEX	DOSEGP	SEX	DOSEGP	Adjustment	Adj P
DOSEGP		2		1	Dunnett-Hsu	0.7460
DOSEGP		3		1	Dunnett-Hsu	0.0229
DOSEGP		4		1	Dunnett-Hsu	0.0008
SEX*DOSEGP	F	2	F	1	Dunnett-Hsu	1.0000
SEX*DOSEGP	F	3	F	1	Dunnett-Hsu	0.9963
SEX*DOSEGP	F	4	F	1	Dunnett-Hsu	0.9344
sex1*DOSEGP	M	2	M	1	Dunnett-Hsu	0.7381
sex1*DOSEGP	M	3	M	1	Dunnett-Hsu	0.0321
sex1*DOSEGP	M	4	M	1	Dunnett-Hsu	0.0015