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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR#: 0054268

MEMORANDUM

Date: December 14, 2006

Subject: **Indoxacarb:** Review of Developmental Neurotoxicity Study - Rat (MRIDs 46749002, 46749003,and 46125302)

PC Code.: 067710
DP Barcode No: D326830

From: Guruva B. Reddy, Veterinary Medical Officer
Registration Action Branch 1
Health Effects Division (HED) (7509P)

To: John Habert, RM 07
Registration Division (7505P)

Through: P.V. Shah, Ph.D., Branch Senior Scientist
Registration Action Branch 1
Health Effects Division (HED) (7509P)

I CONCLUSIONS

The Health Effects Division has evaluated the developmental neurotoxicity study in rat (MRIDs 46749002 46749003, and 46125302) for indoxacarb and provided the Data Evaluation Record (DER). The study is classified as **acceptable/non-guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirements for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (Draft)] due to the pending review of the positive control data. This study satisfies the data gap previously identified.

II ACTION REQUESTED

The Registration Division has requested that the Health Effects Division (HED) review the developmental neurotoxicity study in rat for Indoxacarb (MRIDs 46749002 and 46749003) in

support of registration. E.I. du Pont de Nemours and Company submitted this study is response to HIARC recommendations (July 17, 2000, TXR No. 014241)

CITATION: Barnett, Jr., John F. 2006. Oral (gavage) developmental neurotoxicity study of DPX-KN128 (Indoxacarb) technical in Crl:CD (SD)IGS BR VAF/Plus rats. CR-DDS Argus Division, Protocol No. 104-026. January 24, 2006. MRID 46749002. Unpublished.

Barnett, Jr., John F. 2006. Oral (gavage) developmental neurotoxicity study of DPX-KN128 (Indoxacarb) technical in Crl:CD (SD)IGS BR VAF/Plus rats-Supplement 1. CR-DDS Argus Division, Protocol No. 104-026. January 24, 2006. MRID 46749003. Unpublished.

Stry, James J. 2003. Analysis of selected rat samples from DuPont-10417. DuPont-13478. November 11, 2003. MRID 46125302. Unpublished.

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRIDs 46749002, 46749003 & 46125302) DPX-KN128 (Indoxacarb, 95.47% a.i., Batch No. B-104-026-B-E) was administered once daily to 25 female Crl:CD(SD)IGS BR VAF/Plus rats per dose by gavage at dose levels of 0 (controls), 0.5, 1.0, 1.5 or 3.0 mg/kg bw/day from gestation day 6 (GD 6) through lactation/postpartum day 10 (LD 10). Dams were assessed outside the home cage for abnormal posture, movements, behavior and autonomic dysfunction beginning on GD 6 and continuing through LD 21. Evaluation for adverse clinical signs during parturition, duration of gestation, litter size, live litter size and pup viability at birth was also performed. Maternal behavior was evaluated on post natal days (PND) 0, 4, 7, 13 and 21. On PND 4, litters were standardized to ten pups each (5 males and 5 females when possible). F_1 generation pups were exposed by maternal milk during the maternal postpartum dosage period and were also administered 0, 0.5, 1.0, 1.5 or 3.0 mg/kg/day of DPX-KN128 once daily by gavage from postnatal days 11 through 20. Offspring were then allocated for placement into subsets and were assessed for clinical signs, motor activity, acoustic startle habituation, learning and memory (passive avoidance and water-maze testing), hematology and neurohistology. Positive control data submitted in MRID 46749003. These data were previously reviewed and assessed by Oak Ridge National Laboratory and sent to the U.S. EPA under Task No. 93-2005 (no MRID numbers assigned).

In the maternal animals, a total of 0, 1, 1, 0, and 4 rats were found dead or sacrificed in moribund condition in the 0, 0.5, 1, 1.5 or 3.0 mg/kg/day dose groups, respectively. The rat in the 1 mg/kg/day group was found dead on GD 22 and considered incidental because only one animal was involved. The death on GD 20 in the 0.5 mg/kg group and one of the deaths on LD 10 in the 3 mg/kg group were due to gavage errors while dosing. The other 3 deaths in the 3 mg/kg group on GD 19, GD 20 and LD 3 were considered treatment-related because of accompanying clinical signs.

Treatment-related adverse clinical signs were observed primarily in the same rats from the 3.0 mg/kg/day group that were found dead or were sacrificed. Observations that were the most prominent included: chromorhinorrhea (3/25), dehydration (3/25), soft/liquid feces (2/25), decreased motor activity (2/25), cold to the touch (2/25), hunched posture (2/25), head tilt (2/25), and piloerection (2/25). Other clinical signs only observed in 1/25 dams included rales, lost righting reflex, low carriage and lacrimation. The 3.0 mg/kg/day group also had a statistically significant ($p \leq 0.01$) increase in ataxia (3/22) and abnormal autonomic functions (11/22) during lactation. Autonomic dysfunction evaluation included assessment of lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination, defecation and pupillary response to light.

A statistically significant decrease ($p \leq 0.01$) in mean body weight was observed in the 1.5 and 3.0 mg/kg/day dams on GD 13 but was only decreased by 6 and 8%, respectively, compared to controls. The mean body weight gain in the 3.0 mg/kg/day dams, however, was decreased statistically ($p \leq 0.01$) and toxicologically (23%) on GDs 6-20. A corresponding decrease in food consumption was observed in the 1.5 (12%) and 3.0 (19%) mg/kg/day females on GDs 9-12 compared to controls and decreases of 7 and 17% compared to controls, respectively, during GDs 6-20. Mean body weight was decreased statistically in the 1.5 and 3.0 mg/kg/day dams on LDs 0 and 4 but the decreases were less than 10% when compared to controls. Overall, during gestation, the dams demonstrated a dose dependent decrease in mean body weight, body weight gain and food consumption although it was not always statistically significant. The dams were comparable to controls at all dose levels throughout the lactation period except for early lactation as mentioned above.

Dams in all groups, treated and control, had similar pregnancy rates, number of litters and duration of gestation. There was an increase in the number of stillborn pups (14), in the 3.0 mg/kg/day group, compared to controls (1). The number of pups that died during the first four days was also statistically significantly increased ($p \leq 0.01$) in the 3.0 mg/kg/day group (10.7%) compared to the controls (1%). These pups were from the dams exhibiting signs of toxicity.

The maternal systemic and neurotoxicity LOAEL for DPX-KN 128 (Indoxacarb) in rats is 3.0 mg/kg/day, based on the adverse clinical signs observed, decreased body weight gain and food consumption and mortality. The maternal NOAEL for DPX-KN 128 (Indoxacarb) is 1.5 mg/kg/day.

While a treatment related increase in the number of stillborns and pup mortality in PND 1-4 was observed in the 3.0 mg/kg/day group as stated, the deaths occurring during PND 11-21 were mostly due to gavage errors and not treatment-related. The only treatment-related effect on body weight was a statistically significant decrease ($p \leq 0.05$) observed in the 3.0 mg/kg/day pups at birth. After this time-point, there were no treatment-related effects on the mean body weight, body weight gain or food consumption in the offspring.

Clinical observations, motor activity, acoustic startle habituation, and learning and memory testing were all comparable between the control and treated groups. Mean brain weight, gross

and microscopic examinations and morphometric measurements of the brain were also comparable between the controls and treated groups.

The offspring systemic and neurotoxicity LOAEL for DPX-KN 128 (Indoxacarb) in rats is 3.0 mg/kg/day based on an increased incidence of stillbirths, decreased mean pup body weight at birth and increased pup mortality during PND 1-4. The offspring NOAEL for DPX-KN 128 (Indoxacarb) is 1.5 mg/kg/day.

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

Note: Copy of the DER attached.

DATA EVALUTION RECORD

INDOXACARB/067710
[OPPTS 870.6300]

DEVELOPMENTAL NEUROTOXICITY
MRID NO. 46749002 (main study), 46749003 (positive control data)

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 132-2006

Primary Reviewer:
Dana F. Glass, D.V.M.

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Date: MAY 24 2006

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Signature: Robert H. Ross
Date: MAY 24 2006

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: MAY 24 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

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EPA Reviewer: Guruva Reddy, D.V.M., Ph.D. **Signature:** [signature] 12/14/06
Registration Action Branch 1, Health Effects Division (7509C) **Date:**
EPA Secondary Reviewer: PV Shah, Ph.D. **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 12/18/06

Template version 02/06

TXR#: 0054268

DATA EVALUATION RECORD

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

PC CODE: 067710 **DP BARCODE:** DP 326830; D 364848

TEST MATERIAL (PURITY): DPX-KN128 (95.47%, a.i.)

SYNONYMS: Indoxacarb

CITATION: Barnett, Jr., John F. 2006. Oral (gavage) developmental neurotoxicity study of DPX-KN128 (Indoxacarb) technical in Crl:CD (SD)IGS BR VAF/Plus rats. CR-DDS Argus Division, Protocol No. 104-026. January 24, 2006. MRID 46749002. Unpublished.

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Stry, James J. 2003. Analysis of selected rat samples from DuPont-10417. DuPont-13478. November 11, 2003. MRID 46125302. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company- Wilmington, Delaware

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRIDs 46749002, 46749003 & 46125302) DPX-KN128 (Indoxacarb, 95.47% a.i., Batch No. B-104-026-B-E) was administered once daily to 25 female Crl:CD(SD)IGS BR VAF/Plus rats per dose by gavage at dose levels of 0 (controls), 0.5, 1.0, 1.5 or 3.0 mg/kg bw/day from gestation day 6 (GD 6) through lactation/postpartum day 10 (LD 10). Dams were assessed outside the home cage for abnormal posture, movements, behavior and autonomic dysfunction beginning on GD 6 and continuing through LD 21. Evaluation for adverse clinical signs during parturition, duration of gestation, litter size, live litter size and pup viability at birth was also performed. Maternal behavior was evaluated on post natal days (PND) 0, 4, 7, 13 and 21. On PND 4, litters were standardized to ten pups each (5 males and 5 females when possible). F_1 generation pups were exposed by maternal milk during the maternal postpartum dosage period and were also administered 0, 0.5, 1.0, 1.5 or 3.0 mg/kg/day of DPX-KN128 once daily by gavage from postnatal days 11 through 20. Offspring were then allocated for placement into subsets and were assessed for clinical signs, motor activity, acoustic startle

habituation, learning and memory (passive avoidance and water-maze testing), hematology and neurohistology. Positive control data submitted in MRID 46749003. These data were previously reviewed and assessed by Oak Ridge National Laboratory and sent to the U.S. EPA under Task No. 93-2005 (no MRID numbers assigned).

In the maternal animals, a total of 0, 1, 1, 0, and 4 rats were found dead or sacrificed in moribund condition in the 0, 0.5, 1, 1.5 or 3.0 mg/kg/day dose groups, respectively. The rat in the 1 mg/kg/day group was found dead on GD 22 and considered incidental because only one animal was involved. The death on GD 20 in the 0.5 mg/kg group and one of the deaths on LD 10 in the 3 mg/kg group were due to gavage errors while dosing. The other 3 deaths in the 3 mg/kg group on GD 19, GD 20 and LD 3 were considered treatment-related because of accompanying clinical signs.

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The maternal systemic and neurotoxicity LOAEL for DPX-KN 128 (Indoxacarb) in rats is 3.0 mg/kg/day, based on the adverse clinical signs observed, decreased body weight gain and food consumption and mortality. The maternal NOAEL for DPX-KN 128 (Indoxacarb) is 1.5 mg/kg/day.

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

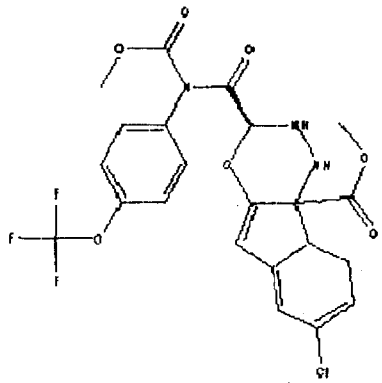
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** DPX-KN128

Description:	Clear liquid at room temperature
Lot/Batch #:	Batch No. B-104-026-B-E (exp. 12/04/09)
Purity:	95.47%, a.i.
Compound Stability:	Stable if refrigerated
CAS No.	173584-44-6



2. **Vehicle and/or positive control:** The test material was formulated with a vehicle, polyethylene glycol (molecular weight 400). The control animals were administered vehicle only by gavage.

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

Species:	Rat
Strain:	Crl:CD (SD)IGS BR VAF/Plus
Age at study initiation:	approximately 68 days old
Wt. at study initiation:	212-256 g
Source:	Charles River Laboratories, Inc. Portage, Michigan
Housing:	Parent animals were housed individually in stainless steel, wire-bottom cages except during cohabitation. Dams and litters were housed together until weaning.
Diet:	Certified Rodent Diet #5002 (PMI Nutrition International, Inc. St. Louis, MO), *ad libitum*
Water:	tap water processed through reverse osmosis, *ad libitum*
Environmental conditions:	Temperature: 64 °F to 79 °F (18 °C to 26 °C) Humidity: 30 to 70% Air changes: 10 air changes/hour Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	5 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: December 26, 2004; End: April 7, 2005

2. **Study schedule:** Prepared formulations of the test material in polyethylene glycol were administered to females by gavage once daily at doses of 0 (vehicle only), 0.5, 1.0, 1.5, or 3.0 mg/kg bw/day beginning on GD 6 through GD 25 for those females that did not deliver or lactation/postpartum day 10 for those that did. Dams were assessed for abnormal posture, movement, behavior and autonomic dysfunction beginning on GD 6 and continuing through LD 21. Dams were evaluated for adverse clinical signs during parturition, duration of gestation, litter size, live litter size and pup viability at birth. Maternal behavior was evaluated on post natal day (PND) 0, 4, 7, 13 and 21. On PND 4, pups were standardized and the litters reduced to ten pups each (5 males and 5 females when possible). Also on PND 4, 20 litters were randomly selected for examination in the study. Then five male and five females from each litter were assigned to one of five subsets. One male and one female were assigned to the following five subsets: (1) PND 21 brain weight and neurohistological evaluations; (2) water maze and passive avoidance; (3) motor activity and acoustic startle habituation; (4) brain weight and neurohistological evaluations at final sacrifice and observations for autonomic dysfunction and abnormal posture/behavior and (5) hematology. F_1 pups were then administered 0, 0.5, 1.0, 1.5 or 3.0 mg/kg/day of the test material by oral gavage once daily from PND 11 to 20. On PND 21, ten litters from each dose group were randomly selected and the male and female pups assigned to Subset 1 were selected for neurohistological evaluation. On PND 21, 40 pups (20/sex) in each dose group assigned to Subset 5 were selected for blood collection. On PND 27, female rats in Subsets 2-4 were checked for vaginal patency and males from the same sets were evaluated for preputial separation on PND 38. On PNDs 22-24 and 30-31, rats in Subset 2 were tested for passive avoidance. Then on PNDs 58-62 and 65-69, rats in Subset 2 were tested in the watermaze. In Subset 3, rats were tested for motor activity on PNDs 13, 17, 21 and between 58-62; they were also tested on PNDs 22 and between 61-63 for acoustic startle habituation. On PNDs 4, 11, 21, 35, 45 and 60, rats in Subset 4 were given a detailed observation for autonomic dysfunction and at sacrifice, ten rats of each sex and dose group in this subset were used in neurohistological evaluation.

3. **Mating procedure:** After acclimation, 150 maternal rats were paired with 150 male breeder rats and cohabitation occurred in the male rat's cage for a maximum of 5 days. Female rats were examined for a copulatory plug and/or spermatozoa in a vaginal smear at the end of this time period. The day either was observed was designated gestation day (GD) 0 and the females were moved into individual housing.

4. **Animal assignment**: The mated parental rats (F_0 generation) were assigned to five dosage groups (See Table 1) using a computer-generated (weight-ordered) randomization procedure based on the weight recorded on GD 0. On day 4 postpartum (PND 4), a computer-generated randomization procedure or a table of random units was used to select pups to be standardized and litters were reduced to ten pups each (five of each sex, when possible). For the selection of the pups into subsets, either computer-generated randomization procedures or a table of random units was used to select pups. Each litter was represented such that all twenty litters in each dose group were represented, an equal number if possible of male and females were in each dose group, and when the numbers of each sex were not equal, a pup was selected from the larger number sex to standardize litter size.

TABLE 1. Study design						
Experimental parameter		Test substance (mg/kg/day)				
		0 (vehicle)	0.5	1.0	1.5	3.0
Maternal animals						
No. of animals assigned		25	25	25	25	25
Offspring						
Subset 1	Brain weight and neurohistology (PND 21)	10/sex	10/sex	10/sex	10/sex	10/sex
Subset 2	Water maze (PND 60 ± 2 and 67 ± 2 Passive avoidance (PND 23 ±1 and 30 ± 1)	20/sex	20/sex	20/sex	20/sex	20/sex
Subset 3	Motor activity (PND 13, 17, 21 and 60 ± 2) Acoustic startle habituation (PND 22 and 63 ± 2)	20/sex	20/sex	20/sex	20/sex	20/sex
Subset 4	Brain wt and neurohistology (circa PND 70) Observed for signs of autonomic dysfunction, abnormal postures/behavior (PND 4, 11, 21, 35, 45 and 60)	10/sex 20/sex	10/sex 20/sex	10/sex 20/sex	10/sex 20/sex	10/sex 20/sex
Subset 5	Hematology (PND 21)	20/sex	20/sex	20/sex	20/sex	20/sex

5. **Dose selection rationale:** The same laboratory performed a dosage range-finding study with the test substance (Argus Protocol 104-025) to obtain the appropriate concentrations. The developmental neurotoxicity protocol was developed in consultation with HED. The registrant proposed doses of 0, 1.0, 1.5, and 3.5 mg/kg/day (draft protocol 104-026, July 02, 2004) were derived from the dose-range finding DNT study MP062 (75% KN128). The sponsor also demonstrated in a perinatal/postnatal reproduction range-finding study (DuPont-10417) in which pregnant female rats were dosed by gavage to DPX-MP602 at doses up to 4 mg/kg/day from gestation Day 7 through the end of lactation (PPD 22), the presence of residues of DPX-MP062 and its metabolite, IN-JT333 in the milk and plasma of lactating females and as well as plasma and fat of nursing offspring. This demonstrates that the offspring were adequately exposed to parent and as well as metabolites via milk (MRID 46125302). However, the Agency and the sponsor agreed to direct dosing of pups in the definitive study. Based on above information the doses of 0, 0.5, 1.0, 1.5 and 3.0 mg/kg/day; for the maternal animals (6G-11L) and direct dosing of pups during lactation (12L-21L) were established.. The oral (gavage) method for treatment was selected because a more precise dosage can be administered by this method compared to the oral feed method, and it represents one possible route of human exposure.

6. **Dosage administration:** All doses were administered once daily to maternal animals by oral gavage on gestation day (GD) 6 through LD 10 or GD 25 in those rats that did not deliver, in a volume of 2 mL/kg of body weight per day. Dosing was based on the most recent body weight determination. Offspring were also treated by the same method on PNDs 11-20.

7. **Dosage preparation and analysis:** Formulations were prepared once by the Haskell Laboratory for Health and Environmental Sciences (Sponsor) by mixing the appropriate amounts of test material with polyethylene glycol and separating them into daily portions prior to shipping. Once arriving at the test center, the formulations were refrigerated at 1 to 8

° C. Prior to daily administration, the container of formulation was removed from the refrigerator and sonicated until all of the solid material was re-solubilized. These formulations were then continuously stirred during dosing. Six samples were taken from the first and last day of dosing. One set of four samples was used for concentration verification and one set of two samples was used for a five-hour stability verification. Two of the concentrations and one of the stability verification samples were shipped back under refrigeration to the Sponsor for analysis. Prior to the initial shipping, the Sponsor took samples of the formulation from the top, middle and bottom of each formulation to check for homogeneity.

Results:

Homogeneity analysis: C.V. = 1 % for all formulations; Homogeneity of indoxacarb in the formulation was evaluated by calculating the coefficient of variation (C.V. = standard of deviation/mean X 100) of the measured concentration in the top, middle and bottom samples of each concentration. A C.V. of less than 10% implies homogeneity.

Stability analysis: C.V. = 1 to 4% of measured; Stability of indoxacarb in the dosing formulations was evaluated by using the mean result of the duplicate concentration verification samples as the baseline for comparing the corresponding results for the room temperature and refrigerated samples.

Concentration analysis: ± 4.3 to 4.8% of nominal; The mean result of the homogeneity samples (top, middle and bottom) before the study start were used as the concentration verification for the test substance at the respective dosing levels.

The analytical data indicate that the mixing procedure was adequate and that the difference between the nominal and actual dosage to the study animals was acceptable. Test substance was not identified in the 0 mg/mL samples.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals**: Female rats were observed at least twice daily for viability with clinical observations weekly during the predosage period. A blind observer examined the rats at approximately the same time outside of their home cage for signs of autonomic dysfunction, abnormal posture, abnormal movement or abnormal behavior patterns and unusual appearance daily, before dosing, beginning on GD 6 and continuing until LD 21 (those delivering) or GD 25 (those not delivering). Autonomic dysfunction observation included an assessment of lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination, defecation and pupillary response to light.

Body weight was recorded weekly during the pre-dose period; on GD 0; daily during dosing; PNDs 11, 14, 17 and 21 and at sacrifice. Food consumption was recorded on GD 0 and daily during dosing but was not tabulated after PND 13 because of the pup's ability to consume the maternal feed at this age.

Dams were also evaluated for duration of gestation, litter size, and pup viability. Maternal behavior was evaluated on PND 0, 4, 7, 13 and 21.

b. Offspring:

i. **Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day) 0. Live pups were counted, sexed, and weighed individually for each litter on postnatal days 0, 4, 7 and daily through the dosing period. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. The F_1 offspring were treated by oral gavage starting on PND 11 and continuing through the day prior to weaning (PND 20). Clinical observations for any results from treatment were performed and recorded between 60 to 120 minutes post-dosing. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration.

On day 4 postpartum using a computer-randomization procedure, litters were standardized to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible). Then a table of random units was used to select 20 litters for examination on study. One male and one female from each litter were assigned to each subset to ensure all 20 litters were represented. The subsets were as follows: (subset 1) day 21 postpartum brain weight and neurohistopathology examination at sacrifice, (subset 2) watermaze and passive avoidance, (subset 3) motor activity and acoustic startle habituation, (subset 4) brain weight and neurohistopathology examination at sacrifice/ assessment of autonomic dysfunction and (subset 5) hematology evaluation.

ii. **Developmental landmarks**: Beginning on postnatal day 38, male offspring in Subsets 2 through 4 were examined daily for preputial separation. Female offspring in these same subsets were examined daily beginning on PND 27 for age of vaginal patency.

iii. **Post-weaning observations:** After weaning on PND 21, offspring were examined at least twice daily for mortality and clinical observations were made weekly. Individual body weight and feed consumption were recorded weekly.

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

a.) **Observation of autonomic dysfunction:** On PND 4 (post-standardization), 11 (prior to dosing), 21, 35, 45 and 60, all rats assigned to Subset 4 were examined outside the home cage in an assessment by a technician unaware of the rat's dosage group. Animals were evaluated for signs of autonomic dysfunction, abnormal posture, abnormal behavior patterns, and unusual appearance. Autonomic dysfunction evaluation consisted of an assessment of lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination, defecation and pupillary response to light. The description of the assessments did not include any neuromuscular assessment (i.e. hind-limb strength), the scoring criteria utilized or ranking of degree of findings.

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b.) **Motor activity testing:** Motor activity was evaluated on PNDs 13, 17 and 21 prior to dosing and on PND 60 ± 2 days using rats in Subset 3. Movements were measured by a passive infrared sensor mounted outside a stainless steel, wire bottomed cage unless it was a preweaning session in which Plexiglass® flooring was used. For 1 hour, the number of movements and time spent in movement were tabulated at each ten-minute interval. The apparatus monitored a rack of up to 32 cages and sensors during each session. Each rat was tested in the same location on the rack across all test sessions with groups counterbalanced across testing sessions and cages.

c.) **Acoustic startle habituation:** Acoustic startle habituation was evaluated on PNDs 22 and 61 to 63 using rats in Subset 3. One male and one female rat from each litter were tested on both days for reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli. The rats were tested in sets of four within a sound attenuated chamber. Each rat was placed in a small cage above a platform containing a force transducer. A microcomputer sampled the output of the force transducer and controlled the test session. During the last minute of the five-minute adaptation period, ten "blank" trials were given to sample the baseline force without a stimulus. The rats were then tested with 30 msec, 120 dB bursts of noise at ten second intervals for 50 trials. An additional ten "blank" trials followed. The peak amplitude and the latency to peak of each response were recorded and the average response in baseline trials was subtracted to calculate the response magnitude. The average response magnitude and the pattern of responses over ten trial blocks were then compared between dose groups.

d). **Learning and memory testing:**

1.) **Passive avoidance:** Passive avoidance testing assessing learning, short-term retention, long-term retention, and hyperactivity was conducted on PNDs 22 to 24 and retested on PNDs 30 to 31 in rats in Subset 2. The testing apparatus consisted of a two-compartment chamber separated by a sliding door. One compartment was fitted with a bright light and Plexiglas floor. The other compartment was fitted with a grid floor to which a one second pulse of mild electric current (1mA) was delivered. For each trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore until it entered the "dark" compartment. The sliding door was then closed, the light turned off and the brief pulse of current delivered to the grid floor. The rat was then removed from the apparatus and put into a holding cage for 30 seconds until the next trial. Trials were repeated until the rat stayed in the "bright" compartment for 60 seconds on two consecutive trials (criterion for learning) or until 15 trials were completed. The latency (time) to enter the dark compartment or the maximum 60-second interval was recorded for each trial. Each rat was tested twice with the sessions separated by one week. Dose groups were compared using the following measures: number of trials to the learning criterion in the first session (overall learning performance); time to enter the "dark" compartment from the "bright" compartment on trial 1 in the first session (activity level and exploratory tendency comparison); time to enter "dark" compartment from the "bright" compartment on trial 2 (short-term retention);

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number of trials to the learning criterion in the second session (long-term retention); and time to enter the "dark" compartment from the "bright" compartment in trial #1 of the second session (long-term retention).

2.) **Watermaze testing**: Watermaze testing for evaluation of overt coordination, swimming ability, learning and memory was conducted in a water filled M-maze on PNDs 58 to 62 and retested on PNDs 65 to 69 on rats in Subset 2. Each rat was tested in a watertight 16-gauge stainless steel modified M-maze filled with water approximately 9 inches deep with a temperature of $21^0C \pm 1^0C$. For each test, the rat was placed at the base of the M-maze stem farthest from the two arms and required to swim to one of the two goals of the M-maze in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm for trial 1 was the incorrect goal for the remaining trials. Rats that didn't make the correct choice within 60 seconds in any trial were guided to the correct goal. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials per session was 15. Latency (in seconds) to choose the correct goal or the maximum 60-second interval and the number of errors (incorrect turns in the maze) were recorded for each trial. Each rat was tested twice with a one-week interval between sessions. Dose groups were compared using the following measures: number of trials to criterion on the first day of testing (overall learning performance); average number of errors for each trial on the first day of testing (overall learning performance); time to reach the correct goal on trial 2 of the first day of testing (short-term retention); number of trials to criterion on the second day of testing (long-term retention); average number of errors on each trial on the second day of testing (long-term retention); and time to reach the correct goal on trial 1 of the second day of testing (long-term retention).

v. **Hematology:** On the day of the scheduled sacrifice (PND 21), non-fasted pups from Subset 5 were used for blood collection. Approximately 1 ml of whole blood was collected from the vena cava. Within one hour, methemoglobin (MetHb) was measured and the remaining blood was sent on ice to the Sponsor. There, the samples were analyzed for erythrocytes (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) on the day they were collected.

2. **Postmortem observations:**

a. **Maternal animals:** Maternal animals were sacrificed by carbon dioxide asphyxiation after completion of the 22-day postpartum period and a gross examination of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Rats not delivering a litter were sacrificed on GD 25 and examined for gross lesions. These rats also had the uteri examined carefully to ensure there were no implantation sites. The uteri and ovaries of the non-pregnant females were retained in neutral buffered 10% formalin. This same procedure was performed on dams with no surviving pups or for those delivering but not selected for

continued observation after PND 4. Any maternal rats found dead or sacrificed because of moribund conditions were examined for the cause of death or cause of condition by gross examination. One maternal control group rat was randomly selected and all tissues retained at necropsy to provide control tissues, if needed.

b. **Offspring**: Pups that died before initial examination were evaluated to determine if they were stillborn or if they died shortly after birth, by immersion of their lungs, and were also given a gross examination for cause of death. On PND 4, all culled pups were sacrificed and gross necropsy was performed on the thoracic, abdominal and pelvic viscera. At postnatal day 21, the pups from each litter assigned to Subset 1 were sacrificed. After sacrifice, they were perfused *in situ* with 10% neutral buffered formalin (NBF). The remaining F_1 rats were sacrificed after all of the postweaning behavioral evaluations.

Selected rats from Subset 4 were euthanized on PND 70 and were also perfusion-fixed in 10% formalin. After dissection at Argus, the heads were shipped to Consultants in Veterinary Pathology, Inc. (CVP) for removal and processing of the brain, gasserian ganglia and eyes. The carcasses were then shipped to the Pathology Associates Division (PAI) of Charles River Laboratories for further dissection and preparation of the slides of spinal cord, peripheral nervous system and skeletal muscle. After the brain was removed and weighed, two linear measurements were made using a Vernier caliper: anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs and anterior to posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole. These measurements were taken by a "blinded" participant.

After the brain measurements, the brains were sliced and the slices processed within five cassettes on a Citadel tissue processor and embedded in paraffin. Brains from all dose groups were processed to this stage to avoid the potential for shrinkage or swelling from prolonged fixation but only the 0 and 3.0 mg/kg/day juvenile rats were used for histopathological examination. The adult rats from the 0 and 3.0 mg/kg/day groups had the gasserian ganglia and associated trigeminal nerve tissue removed and embedded. Sections of the spinal cord and the sciatic nerve were also processed. In the juvenile rats, the brain sections were stained with hematoxylin and eosin (H&E) and with loxol fast blue/cresyl violet (LFB/CV). In the adult rats, the brain sections as well as sections of the gasserian ganglia, spinal cord and nerve roots were stained with H&E, LFB/CV and with the Bielschowsky's technique. The block lists were as follows:

Parrafin-embedded tissue:

Block 1: Coronal slice through the cerebrum at the level of the optic chiasm
Block 2: Coronal slice through the cerebrum at the level of the infundibulum
Block 3: Coronal slice through the cerebrum at the level of the mammillary bodies
Block 4: Coronal slice through the middle of the cerebrum
Block 5: Multiple sections including the olfactory bulbs
Block 6: Longitudinal sections of the Gasserian ganglia and associated trigeminal nerves
Block 7: Sections of the eyes

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Block 8: Longitudinal sections of the dorsal root ganglia and spinal nerve roots
Block 9: Cross and longitudinal sections of the spinal cord
Block 10: Cross and longitudinal sections of the gastrocnemius muscle

Glycol methacrylate-embedded tissue:
Block 11: Longitudinal section of the sciatic nerve plus cross section of the sciatic and tibial nerves
Block 12: Longitudinal sections of the common peroneal, tibial and sural nerves

Eleven linear microscopic measurements were taken from each brain of the juvenile and adult rats in the control and highest dose group. These eleven measurements represent six regions. The measurements were taken by a study pathologist blinded to the group identification, using a calibrated ocular micrometer. The eleven measurements represented six regions and were as follows:

1. Thickness of the frontal cortex
2. Thickness of the parietal cortex
3. Diagonal width of the striatum
4. Thickness of the corpus callosum just lateral to its midpoint
5. Thickness of the hippocampal gyrus
6. Maximum height of the hippocampal gyrus

The CHECKED (X) tissues were evaluated for adult offspring.[a]

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Cross and longitudinal sections of the sciatic nerve
X	Center of cerebrum		
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve
	SPINAL CORD	X	Peroneal nerve
X	Cervical swelling	X	Lumbar dorsal root fibers
X	Lumbar swelling	X	Lumbar dorsal root ganglion
	OTHER	X	Lumbar ventral root fibers
X	Gasserian ganglion	X	Cervical dorsal root ganglion
X	Trigeminal nerves	X	Cervical dorsal root fibers
X	Optic nerve	X	Cervical ventral root fibers
X	Eyes	X	Skeletal muscle

[a] brain weights and neurohistopatholgy was done on PND21 and adult offspring

D. DATA ANALYSIS:

1. **Statistical analyses:** Data were tabulated, summarized and/or statistically analyzed using the following programs: Argus Automated Data Collection and Management System, The Vivarium Temperature and Relative Humidity Monitoring System, MicroSoft ® Excel (part of Microsoft Office 97/2000/XP), Quattro Pro 8 and/or the SAS System (version 6.12).

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Variables with interval or ratio scales of measurement, such as body weight, food consumption values, latency and errors per trial scores in behavioral tests and percent mortality per litter were analyzed using the parametric systems and began with Bartlett's test of homogeneity of variance. A non-significant result ($p > 0.001$) indicated that an assumption of homogeneity of variance was appropriate and the data were compared using the analysis of variance test. If that test was significant ($p \leq 0.05$), the treated groups were compared with the control group using Dunnett's test. If Bartlett's test was significant ($p \leq 0.001$), the analysis of variance test was not appropriate and nonparametric analysis was used. When 75% or fewer of the scores in all the groups were tied, the Kruskal-Wallis test was used and in the event of a significant result ($p \leq 0.05$), Dunn's test was used to compare the treated groups to the controls. When more than 75% of the scores were tied, Fisher's Exact test was used to compare the proportion of ties in the dose groups. Variables having graded or count scores (i.e. litter sizes) were analyzed using the nonparametric methods. Clinical observation incidence data were analyzed as contingency tables using the variance test for homogeneity of the binomial distribution.

Clinical pathology data were analyzed first with Levene's test for homogeneity and the Shapiro-Wilk test for normality. If these were not significant, they a one-way analysis of variance followed by a Dunnett's test was used. If they were significant, then the Kruskal-Wallis test was followed with Dunn's test.

Using the motor activity and acoustic startle data, BioStat Consultants, Inc. evaluated the values and performed statistical analysis both within each session and across sessions. The objective of the analysis within sessions was to evaluate treatment group responses across the intervals and across sessions to evaluate treatment group responses across the different post-treatment sessions. Statistical methodology used for analyzing motor activity and acoustic startle data is attached in Appendix B.:

2. **Indices**:

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

      Gestation index= number of rats with live offspring/number of pregnant rats

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

      Viability index = number of live pups on day 4 (pre-culling) postpartum/number of liveborn pups on day 0 postpartum.

      Lactation index = number of live pups on day 10 postpartum/number of live pups on day 4 (post-culling) postpartum

3. **Positive and historical control data:** Positive control data conducted by Argus Laboratories were submitted concurrently (MRID 46749003). These data are under review. Watermaze historical control data from the reporting laboratory was included in the study

report for the time period from 1992 through 2005. Summaries of the data are included in Appendix A.

II. RESULTS:

A. PARENTAL ANIMALS:

1. Mortality and clinical and functional observations:

A total of 0, 1, 1, 0, and 4 rats were found dead or were sacrificed in the 0, 0.5, 1, 1.5 and 3.0 mg/kg/day dose groups, respectively. The rat in the 1 mg/kg/day group was found dead on GD 22 and was considered incidental because only one dam was involved. At necropsy, this dam had a litter of 17 fetuses that were developmentally normal. The death on GD 20 in the 0.5 mg/kg group and one of the deaths on LD 10 in the 3 mg/kg group were due to a gavage incident while dosing and both had normal litters. The other 3 deaths in the 3 mg/kg group were treatment-related because of the accompanying signs of neurotoxicity.

One dam sacrificed on LD 3 in the 3.0 mg/kg/day group had displayed ataxia (LD 1-3), hunched posture (LD 2-3), lost righting reflex, decreased motor activity and emaciation (LD 3). The dam had a litter of 14 pups with 5 found dead and 9 sacrificed with the dam. The second dam, found dead in the 3.0 mg/kg/day group on GD 20, had soft/liquid feces (GD 17-19), pale extremities and dehydration (GD 18-19) and ataxia, hunched posture, cold to the touch and head tilt (GD 19). The dam had a litter of 12 dead pups which appeared normal. The last dam found dead in the 3.0 mg/kg/day group on GD 19 also had clinical signs of chromorhinorrhea (GD 14), cold to touch and soft feces (GD 16-18), hunched posture (GD 17), and decreased motor activity, head tilt, low carriage and piloerection (GD 18). The dam had 2 resorptions and 15 dead fetuses normal for their developmental age.

The 3.0 mg/kg/day group had a statistically significant ($p \leq 0.01$) increase in ataxia (3/22) and abnormal autonomic functions (11/22) during the lactation period. There was a significant increase ($P \leq 01$) in the number of rats in the 0.5 mg/kg/day group exhibiting abnormal autonomic function (8/6)during the lactation period and also soft or liquid feces. Since these effects were not seen at the two mid-doses, the effects seen at 0.5 mg/kg/day is not considered treatment-related effects. These results are summarized in Table 2. The severity of the clinical observations was not included in the study report. The registrant's email (7/14/06) provided Argus Research Laboratories SOPs for conducting an autonomic function evaluation which suggests to mark the findings on a scale of slight, moderate or extreme when noticed (Argus Protocol 104-026).

TABLE 2. Maternal mortality and clinical/functional observations[a]					
Observation	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Mortality: Found dead Moribund sacrifice	0 0 0	1 1 (GD 20) 0	1 1 (GD 22) 0	0 0 0	4 3 (GD 19, 20 and LD10) 1 (LD3)
Gestation					
No. of animals	25	25	25	25	25
Chromorhinorrhea	1/1[b] (14)[c]	0/0	1/1 (19)	1/1 (14)	3/3 (14, 19, 20)
Dehydration	0/0	0/0	2/1 (17,19)	1/1 (17)	5/3 (17, 18, 21)
Soft/liquid feces	0/0	0/0	1/1 (6)	0/0	6/2 (16-18)
Decreased motor activity	0/0	0/0	3/1 (19-21)	0/0	2/2 (18, 21)
Cold to the touch	0/0	0/0	0/0	0/0	4/2 (16-18, 19)
Hunched posture	0/0	0/0	0/0	0/0	2/2 (17, 19)
Head tilt	0/0	0/0	0/0	0/0	2/2 (18, 19)
Piloerection	0/0	0/0	0/0	0/0	2/2 (18, 19)
Rales	0/0	1/1 (19)	0/0	0/0	1/1 (21)
Lost righting reflex	0/0	0/0	0/0	0/0	1/1 (21)
Low carriage	0/0	0/0	0/0	0/0	1/1 (18)
Lacrimation	0/0	0/0	0/0	0/0	1/1 (21)
Lactation					
No. of animals	21	23	23	25	22
Autonomic functions were not normal	0/0	8/6** (1, 2, 3, 4, 5, 11)	3/3	5/4	17/11** (1-3, , 2-3, 3, 4, 6-9, 7, 10, 11, 12, 20)
Soft/liquid feces	0/0	8/3 (3,6,7)	2/2	2/2	4/4 (10, 12, 17, 20)
Ataxia	0/0	0/0	0/0	0/0	7/3** (0,1-3 for 2 rats)
Chromorhinorrhea	1/1 (17)	1/1 (7)	0/0	1/1 (16)	3/3 (18, 19 for 2 rats)
Hunched posture	0/0	0/0	0/0	7/1 (4-10)	6/3 (1,2,3)

[a] Data obtained from pages 108-111 and 130-146 in MRID 46749002.
[b] N/N = total number of observations/number of rats with observation
[c] Number in parenthesis is the day observation occurred
** Statistically different ($p \leq 0.01$) from the control.

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.
A statistically significant ($p \leq 0.01$) decrease in mean maternal body weight (within 10% of control weight) was observed at most time-points in gestation in the 3.0 mg/kg/day rats. Weight gain in the 3.0 mg/kg/day dams during GDs 6-20 was statistically ($p \leq 0.01$) and toxicologically decreased (23%) compared to that of controls. Body weight was statistically ($p \leq 0.01$) decreased in the dams treated with 1.5 and 3.0 mg/kg/day during lactation days 0-3 (less than 10%) and not considered toxicologically significant.

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During GDs 9-12, food consumption was decreased significantly ($p \leq 0.05$) compared to controls in the 1.5 mg/kg/day group females. Food consumption in the 3.0 mg/kg/day females was statistically significantly decreased for most of gestation, down from 14-23% of control levels. Food consumption was not affected in any of the maternal animals at any dose during lactation.

TABLE 3. Mean (±SD) maternal body weight and food consumption [a]

Observation/ Study week	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Gestation					
Mean body weight (g) Gestation day 6	272.3 ± 12.3	268.2 ± 12.8	267.8 ± 15.3	265.0 ± 14.4	267.4 ± 15.4
Mean body weight (g) Gestation day 13	312.2 ± 18.0	303.9 ± 16.4	300.7 ± 16.8	293.2** ± 17.6 (6)[b]	288.4** ± 21.9 (8)
Mean body weight (g) Gestation day 20	388.2 ± 23.7	379.4 ± 25.0	374.3 ± 29.8	371.1 ±27.2	355.4** ± 29.5 (8)
Mean weight gain (g) Gestation days 6-20	115.8 ± 14.1	111.3 ± 17.8	106.5 ± 28.0	106.1 ± 18.1	89** ± 25.1 (23)
Mean food consumption (g/day) Gestation days 0-6	22.4 ± 2.1	22.0 ± 2.3	21.9 ± 2.6	21.2 ± 2.4	21.6 ± 2.0
Mean food consumption (g/day) Gestation days 9-12	23.6 ± 2.5	23.5 ± 3.0	22.4 ± 2.6	20.8** ± 3.0 (12)	19.2** ± 4.7 (19)
Mean food consumption (g/day) Gestation days 15-18	25.2 ± 2.1	25.1 ± 2.3	24.6 ± 7.5	24.4 ± 3.8	19.4** ± 6.1 (23)
Mean food consumption (g/day) Gestation days 6-20	24.0 ± 2.0	23.9 ± 2.2	22.9 ± 3.0	22.2* ± 2.6 (7)	19.8** ± 2.8 (17)
Mean food consumption (g/day) Gestation days 0-20	23.6 ± 2.0	23.3 ± 2.1	22.6 ± 2.3	21.9* ± 2.3 (7)	20.4** ± 2.3 (14)
Lactation					
Mean body weight (g) Lactation day 0	298.7 ± 19.6	291.0 ± 15.2	289.7 ± 19.1	282.9* ± 18.1	275.2** ± 23.4 (8)
Mean body weight (g) Lactation day 4	298.1 ± 16.5	294.9 ± 21.3	292.1 ± 20.5	283.7* ± 20.2	280.1* ± 26.7 (6)
Mean body weight (g) Lactation day 7	310.7 ± 21.3	308.3 ± 19.0	304.9 ± 25.3	299.4 ± 20.0	301.0 ± 24.8
Mean body weight (g) Lactation day 14	336.8 ± 20.3	334.2 ± 21.2	331.3 ± 27.0	324.2 ± 15.3	329.8 ± 22.5
Mean body weight (g) Lactation day 21	341.8 ± 19.4	338.1 ± 23.8	338.4 ± 18.2	332.0 ± 16.3	337.9 ± 20.9
Mean weight gain (g) Lactation days 0-21	41.8 ± 9.1	47.4 ± 18.9	46.8 ± 11.6	50.4 ± 14.3	58.4** ± 12.2
Mean food consumption (g/day) Lactation days 7-11	43.6 ± 5.1	43.5 ± 5.6	44.6 ± 6.2	44.0 ± 5.7	44.4 ± 7.2
Mean food consumption (g/day) Lactation days 0-13	47.1 ± 5.3	46.8 ± 5.7	47.7 ± 6.1	47.2 ± 5.5	47.6 ± 6.8

[a] Data obtained from pages 114-122 in MRID 46749002.
[b] Number in parentheses is percent decreased from control, calculated by the reviewer.
* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$

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3. **Reproductive performance:** Delivery observations were based on the 21-25 rats that survived the gestation period. The number of dams delivering litters, the duration of gestation and the gestation index was similar between all groups, treated and control. Thus the gestation index was 100% for each group. Results for the maternal animals are summarized in Table 4.

TABLE 4. Reproductive function[a]

Observation	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Number of dams pregnant	21	24	24	25	25
Number included in analysis- delivered a litter	21	23[b]	23	25	23[b]
Mean (±SD) gestation duration (days)	22.6 ± 0.5	22.6 ± 0.5	22.6 ± 0.5	22.6 ± 0.5	22.5 ± 0.5
Gestation index[c]	21/21 (100%)	23/23 (100%)	23/23 (100%)	25/25 (100%)	23/23 (100%)

[a] Data obtained from Table B11, page 124 in MRID 46749002.

[b] Excludes dams found dead during gestation

[c] Number of rats with live offspring/number of pregnant rats

4. **Maternal postmortem results**: Treatment-related effects were not identified in any of the dams at necropsy. Animals that appeared to have died due to dosing errors had perforations in the esophagus and/or fluid in the trachea or lungs.

B. OFFSPRING:

1. **Viability and clinical signs**: Litter size and viability (survival) results from pups during lactation are summarized in Table 5. The number of stillborn pups was greatly increased in the 3.0 mg/kg/day group compared to controls during PND 1-4. This decreased in viability was primarily due to one litter having 13 stillborn pups. A statistical increase ($p<0.05$, $p<0.01$) in the number of male pups compared to females was reported in the 0.5 and 1.5 mg/kg/day group, but it was not dose responsive.

   There was significant increase ($p \leq 0.05$ or 0.01) in mortality in male pups at 0.5 and 1.5 mg/kg/day, respectively, compared to controls during PND 11-21. These increase in mortality was primarily due to gavage errors. The number of males died due to gavage errors were 1, 4, 1, 6 and 0 in each of 0, 0.5, 1.0, 1.5 and 3.0 mg/kg/day groups, respectively; and the number of females died due to gavage errors were 1, 3, 5, 3, and 0 each in control, 0.5, 1.0, 1.5 and 3.0 mg/kg/day group respectively. The total number of males died including gavage errors were 3, 15, 1, 10, and 1 in each of the 0, 0.5, 1.0 and 3.0 mg/kg/day, respectively. The significantly increased number of deaths in the 0.5 and 1.5 mg/kg/day dosage group male rats were not considered treatment-related since the response was not dose-related and was due to gavage errors.

TABLE 5. Litter size and viability[a]					
Observation	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Total number born	299	342	326	349	335
Number born live	298	339	325	349	321
Number born dead	1	3	1	0	14
Sex Ratio Day 0 (% %)	46.3 ± 12.6	55.7* ± 12.6	54.9 ± 10.2	59.6** ± 14.5	52.4 ± 17.2
# Deaths Days 1-4 (%)	3/297 (1.0)	5/338 (1.5)	5/322 (1.6)	7/349 (2.0)	34/318** (10.7)
# Deaths Days 5-7 (%)	3/204 (1.5)	0/221 (0)	0/226 (0)	0/232 (0)	1/194 (0.5)
# Deaths Days 8-10 (%)	1/201 (0.5)	0/221 (0)	1/226 (0.4)	0/232 (0)	0/193 (0)
# Deaths Days 11-21 (%) Males	3/93 (3.2)	15/99 (15.2)**	1/99 (1.0)	10/99 (10.0)**	1/91 (1.0)
# Deaths Days 11-21 (%) Female	5/100 (5.0)	4/98 (4.0)	6/98 (6.1)	7/94 (7.4)	3/92 (3.3)
# Deaths Days 11-21 (%) Males + Females	8/193 (4.1)	19/197 (9.6)	7/197 (3.6)	17/193 (8.8)	4/183 (2.2)
Surviving pups/litter mean ± SD:					
Day 0	14.2 ± 1.6	14.7 ± 2.9	14.1 ± 1.7	14.0 ± 2.0	14.0 ± 3.0
Day 4 (pre-culling)	14.0 ± 1.6	14.5 ± 2.7	13.8 ± 1.7	13.7 ± 1.8	12.3 ± 5.1
Day 4 (post-culling)	9.7 ± 0.8	9.6 ± 0.8	9.8 ± 0.4	9.3 ± 0.9	8.4 ± 3.4
Day 7	9.6 ± 1.0	9.6 ± 0.8	9.8 ± 0.4	9.3 ± 0.9	8.4 ± 3.4
Viability index (%) (N/N)	98.6 (294/298)	98.2 (333/339)	97.5 (317/325)	98.0 (342/349)	88.5** (284/321)
Lactation index	98.0 (200/204)	100.0** (221/221)	99.6** (225/226)	100.0** (232/232)	99.5 ** (193/194)

[a] Data obtained from pages 125-126 and 236-242 in MRID 46749002.
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

No treatment-related clinical observations on offspring were observed. The two most common observations during the ten days of lactation were cold to touch and dehydration. The cold to touch was observed in both the 1.0 mg/kg/day group (in 3 litters) and the 3.0 mg/kg/day group (in 4 litters) but was not in a dose-related tendency as none were observed in the 1.5 mg/kg/day group. Dehydration was observed equally in the controls as well as the 3.0 mg/kg/day group.

2. **Body weight**: A statistically significant ($p \leq 0.05$) decrease in mean pup body weight was observed in the 3.0 mg/kg/day group compared to controls on Day 0. This was comparable in all groups by Day 7. Data are presented in Table 6.

TABLE 6. Mean (± SD) pup body weight in PND 0-7(g)[a]					
Postnatal day	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
	Males/Females				
Day 0	6.4 ± 0.5	6.4 ± 0.6	6.5 ± 0.4	6.4 ± 0.6	5.9* ± 0.6
Day 4 (pre-culling)	9.2 ± 0.9	9.3 ± 1.8	9.6 ± 1.1	9.3 ± 1.4	8.8 ± 1.2
Day 4 (post-culling)	9.2 ± 0.9	9.4 ± 1.8	9.7 ± 1.0	9.3 ± 1.4	8.8 ± 1.2
Day 7	14.2 ± 2.2	14.4 ± 3.1	14.7 ± 2.4	14.6 ± 2.4	13.8 ± 2.4

[a] Data obtained from page 127 in MRID 46749002.
* Statistically different from control, $p \leq 0.05$
N = 298-349

There were no treatment-related effects on body weight or food consumption in either the male or female offspring after the decrease seen on Day 0. See Table 7. Occasional statistical increases were observed primarily in the 3.0 mg/kg/day males when compared to controls but they were sporadic, less than 10% and not observed in a dose responsive manner. Absolute feed consumption increased significantly ($P < 0.05 - 0.01$) in the 0.5, 1, and 3 mg/kg/day day females on PND 21-76, and also on PND 21 – 28 and PND 69 -76 in high dose females, compared to controls. Feed consumption in females was not considered treatment-related,because it lacked dose response and the changes were sporadic.

TABLE 7. Mean (± SD) pup body weight (g) and food consumption (g/day)[a]					
Postnatal day	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Males					
Mean body wt					
Day 11	23.5 ± 3.6	22.6 ± 5.3	24.1 ± 4.0	23.9 ± 3.8	23.5 ± 4.4
Day 16	35.0 ± 4.7	34.6 ± 6.8	35.9 ± 5.1	35.9 ± 4.3	35.7 ± 5.4
Day 21	47.2 ± 6.7	48 ± 9.6	49.1 ± 7.4	49.1 ± 6.5	48.1 ± 7.8
Day 42	195.4 ± 22.4	199.9 ± 30.1	207.6 ± 27.7	203.0 ± 19.6	206.6 ± 25.1
Day 76	444.5 ± 42.5	435.4 ± 46.5	451.4 ± 40.7	444.0 ± 36.0	459.7 ± 35.4
Mean body wt change					
Days 11-15	+ 9.4 ± 1.5	+ 9.2 ± 2.4	+ 9.4 ± 1.4	+ 9.9 ± 1.7	+ 9.9* ± 1.5 (105)
Days 11-21	+ 23.8 ± 3.5	+ 25.0 ± 4.9	+ 24.9 ± 3.7	+ 25.2 ± 3.5	+ 24.6 ± 3.8
Days 35-42	+ 57.8 ± 6.6	+ 60.9* ± 7.5	+ 62.6** ± 7.7	+ 62.2** ± 5.4	+ 63.1** ± 7.2 (109)
Days 21-76	+ 396.7 ± 37.6	+ 385.2 ± 39.7	+ 401.2 ± 36.0	+ 394.0 ± 32.6	+ 408.0 ± 34.9
Food consumption					
Days 21-28	10.8 ± 1.4	11.2 ± 1.9	12.0** ± 1.6	11.8** ± 1.5	12.4** ± 1.7 (115)
Days 49-56	26.8 ± 2.3	28.0 ± 2.7	28.4** ± 3.0	27.5 ± 1.8	28.3** ± 3.5 (106)
Days 69-76	28.2 ± 3.0	29.0 ± 3.5	30.0 ± 2.8	28.2 ± 2.7	29.9 ± 3.1
Days 21-76	23.2 ± 2.1	23.7 ± 2.1	24.4 ± 2.1	23.5 ± 1.3	25.1** ± 2.1 (108)
Females					
Mean body wt					
Day 11	23.1 ± 3.8	22.1 ± 5.0	23.2 ± 3.6	23.0 ± 3.7	22.8 ± 4.0
Day 16	34.8 ± 4.5	33.6 ± 6.4	34.2 ± 4.5	34.3 ± 4.2	34.5 ± 4.6
Day 21	46.8 ± 5.7	46.1 ± 8.6	47.1 ± 6.6	47.1 ± 6.3	46.7 ± 6.7
Day 42	153.9 ± 14.7	153.3 ± 19.3	157.4 ± 16.3	155.8 ± 15.7	156.9 ± 16.6
Day 76	246.8 ± 20.4	254.1 ± 27.9	260.4 ± 22.9	252.7 ± 22.5	265.1 ± 24.8
Mean body wt change					
Days 11-15	+ 9.4 ± 1.6	+ 9.2 ± 2.0	+ 8.8* ± 1.3	+ 9.3 ± 1.5	+ 9.4 ± 1.3
Days 11-21	+ 23.4 ± 2.8	+ 24.0 ± 4.2	+ 23.9 ± 3.3	+ 24.1 ± 3.2	+ 23.8 ± 3.4
Days 35-42	+ 33.8 ± 6.2	+ 34.0 ± 5.2	+ 35.6 ± 5.5	+ 34.5 ± 5.0	+ 34.7 ± 6.4
Days 21-76	+ 198.4 ± 19.8	+ 206.9 ± 25.5	+ 212.7 ± 21.1	+ 204.4 ± 20.4	+ 216.1 ± 24.3
Food consumption					
Days 21-28	10.8 ± 1.4	10.7 ± 1.5	11.4 ± 1.3	11.3 ± 1.0	11.6** ± 1.3 (107)
Days 49-56	19.1 ± 2.7	20.0 ± 2.2	19.4 ± 1.9	19.7 ± 2.6	20.2 ± 3.1
Days 69-76	18.7 ± 2.1	20.2 ± 2.6	19.8 ± 2.3	18.2 ± 1.6	20.5* ± 3.4 (110)
Days 21-76	17.0 ± 1.5	18.2* ± 1.7	18.3* ± 1.6	17.7 ± 1.4	19.0** ± 1.9 (112)

[a] Data obtained from pages 245-253 and 255 in MRID 46749002.
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

3. **Developmental landmarks:**

Sexual maturation: Sexual maturation was normal for the control and treated male and female rats. Preputial separation was complete by 46.2, 46.0, 45.1, 44.9 and 45.2 days in the control, 0.5, 1.0, 1.5 and 3.0 mg/kg/day groups, respectively. The average day of preputial separation was significantly reduced , ($p \leq 0.05$) in the 1 mg/kg/day dosage group, as compared to the control group value. This decrease was not dosage-dependent, and therefore, was not considered to be treatment-related. Vaginal patency was attained by 32.1, 32.2, 32.2, 32.6 and 32.3 days in the control, 0.5, 1.0, 1.5 and 3.0 mg/kg/day groups, respectively. The data are presented in Table 8.

TABLE 8. Mean (±SD) age of sexual maturation (days) [a]

Parameter	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
N (M/F)	56/58	46/53	57/51	54/53	56/55
Preputial separation (M)	46.2 ± 3.0	46.0 ± 4.2	45.1* ± 4.0	44.9 ± 2.7	45.2 ± 2.5
Vaginal patency (F)	32.1 ± 2.3	32.2 ± 2.7	32.2 ± 3.0	32.6 ± 2.0	32.3 ± 2.8

[a] Data obtained from Table C13, page 257 in MRID 46749002.
* significantly different than controls, ($p \leq 0.05$)

4. **Behavioral assessments:**

a. **Clinical observations:** No treatment-related effects were observed in either the male or female offspring during the assessments looking for autonomic dysfunction, or abnormal behavior, posture, or movements. Ungroomed coats were observed in 4/91 male rats in the 3.0 mg/kg/day group compared to 0/93 of the controls during the dosage period but were not observed at any other time. Other parameters noted were observed equally in the controls and treated groups making them toxicologically insignificant.

b. **Motor activity:** Total activity data are presented in Table 9. The activity level in both males and females followed the same pattern by increasing from PNDs 13 to 17 and from days 21 to 60 with a decrease in activity from PNDs 17 to 21. The activity levels were consistent between the dose groups and controls.

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TABLE 9. Mean (±SD) motor activity data (total number of movements for session) [a]					
Test day	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Males					
PND 13	321.3 ± 172.2	328.2 ± 175.3	316.5 ± 97.7	350.3 ± 162.9	336.3 ± 165.1
PND 17	536.5 ± 165.1	480.1 ± 231.3	465.5 ± 199.4	538.3 ± 138.7	560.1 ± 144.8
PND 21	450.9 ± 142.2	438.3 ± 218.7	411.7 ± 186.7	392.6 ± 163.6	491.7 ± 208.3
PND 60	687.4 ± 149.2	719.8 ± 117.1	571.5 ± 155.3	596.0 ± 149.9	683.3 ± 164.3
Females					
PND 13	373.8 ± 187.3	394.4 ± 195.9	410.6 ± 163.2	377.0 ± 181.5	426.3 ± 170.2
PND 17	592.4 ± 221.9	569.1 ± 193.8	631.6 ± 183.4	589.5 ± 146.9	649.2 ± 136.7
PND 21	471.7 ± 190.8	467.8 ± 204.3	538.8 ± 145.2	407.4 ± 164.3	548.3 ± 179.3
PND 60	802.4 ± 102.1	821.4 ± 94.5	807.8 ± 98.6	782.4 ± 144.3	796.4 ± 95.7

[a] Data obtained from Table F1 on pages 567-574 in MRID 46749002.
N = 16-20

c. **Acoustic startle reflex habituation:** No treatment-related effects were observed in any of the rats tested either on PND 22 or 63 ± 3. The peak amplitude and latency to peak were similar between the controls and treated animals. An appropriate decrease in response (habituation) across the blocks occurred. The amplitude and habituation data are presented in Tables 10 and 11. In males on PND 22, there was a decrease in startle amplitude relative controls across the blocks 2 to 5. The changes in other doses levels did not show a dose-related response. On PND 63 similar results were seen at the 3 mg/kg/day dose. There is a dose-related effect starting at 1 - 3 mg/kg/day dose levels, whereas at the lowest dose (0.5 mg/kg/day) there is increase in amplitude, relative to controls. In females, however, the response was inconsistent among various dose levels. At 0.5, 1, and 3 mg/kg/day dose levels, there was an increase in amplitude while at 3 mg/kg/day amplitude increased on PDN 22. At PND 63, this amplitude increased at all dose levels relative to controls but was not dose-related. decreased 8, 1, 8, and 20%, in 0.5, 1.0, 1.5 and 3.0 mg/kg/day dose groups, respectively; while in females the response was −19, +18, -13 and +20%, respectively.

Auditory startle reflex latency data are presented in Table 11. The results of auditory startle reflex latency did not show substantial difference in both males and females, and no habituation was reflects by the data.

TABLE 10. Auditory Startle Reflex Amplitude (g) Data (mean ± S.D.)[a]

BLOCK		Dose (mg/kg/day)				
		0	0.5	1.0	1.5	3.0
Males						
PND 22	1	17.84 ± 11.50	16.41 ± 8.32	19.10 ± 9.21	16.90 ± 9.27	17.11 ± 8.15
	2	13.30 ± 8.54	13.73 ± 10.15	13.00 ± 7.99	10.47 ± 7.40	11.54 ± 6.16
	3	11.77 ± 10.96	13.29 ± 11.76	11.52 ± 7.23	9.97 ± 7.67	9.88 ± 5.74
	4	13.03 ± 10.68	10.15 ± 8.04	12.88 ± 8.63	13.01 ± 7.63	8.56 ± 4.08
	5	16.98 ±13.55	13.49 ± 10.96	15.72 ± 10.39	16.54 ± 14.59	11.55 ± 8.64
	Mean	**14.57**	**13.41 (-8%)**	**14.44 (-1.0%)**	**13.38 (-8.0%)**	**11.73 (-20%)**
PND 63±2	1	99.14 ± 48.86	109.99 ± 76.10	105.00 ± 56.64	91.15 ± 91.70	84.45 ± 42.91
	2	73.38 ± 60.47	76.11 ± 52.96	66.82 ± 73.54	67.20 ± 104.79	56.06 ± 33.46
	3	56.16 ± 57.94	55.53 ± 34.43	49.58 ± 47.65	41.49 ± 23.67	41.71 ± 23.67
	4	50.34 ± 35.13	49.84 ± 28.32	43.65 ± 47.62	34.80 ± 23.70	38.16 ± 30.92
	5	50.23 ± 45.50	44.76 ± 32.18	33.13 ± 28.10	32.88 ± 21.56	34.11 ± 18.80
	Mean	**65.85**	**67.21 (+12%)**	**59.63 (-9%)**	**53.50 (-19%)**	**50.9 (-23%)**
Females						
PND 22	1	17.74 ± 9.64	21.41 ± 11.73	18.75 ± 11.10	13.00 ± 7.45	19.66 ± 11.97
	2	12.03 ± 6.85	14.82 ± 9.27	12.07 ± 8.82	10.15 ± 7.11	15.22 ± 10.53
	3	10.62 ± 7.39	13.05 ± 8.22	10.99 ± 6.56	11.07 ± 12.50	14.35 ± 10.36
	4	9.92 ± 6.13	11.58 ± 8.63	14.82 ± 10.38	10.21 ± 14.47	14.16 ± 9.86
	5	10.82 ± 6.51	12.21 ± 8.64	15.32 ± 11.39	8.76 ± 8.82	14.71 ± 13.42
	Mean	**12.23**	**14.61 (+19%)**	**14.39 (+18%)**	**10.64 (-13%)**	**15.62 (+28%)**
PND 63±2	1	38.58 ± 26.55	47.69 ± 26.34	57.10 ± 31.87	39.96 ± 30.89	54.49 ± 26.83
	2	20.92 ± 11.86	23.87 ± 19.88	31.17 ± 29.39	31.13 ± 30.32	28.32 ± 22.63
	3	22.37 ± 16.22	22.40 ± 13.58	22.69 ± 11.36	18.14 ± 14.87	23.33 ± 14.19
	4	18.69 ± 15.72	20.99 ± 14.77	26.87 ± 20.21	17.26 ± 13.74	21.33 ± 15.45
	5	14.50 ± 9.34	22.43 ± 16.68	22.80 ± 22.32	17.09 ± 13.79	21.72 ± 16.78
	Mean	**23.01**	**27.68 (+20%)**	**32.13 (+40%)**	**24.71 (+7%)**	**29.84 (+30%)**

[a] Data were obtained from Tables F2 and F3, pages 575 - 578 in MRID 46749002
N= 16-20

TABLE 11. Auditory Startle Reflex Latency (msec) Data (mean ± S.D.)[a]						
BLOCK		**Dose (mg/kg/day)**				
		0	**0.5**	**1.0**	**1.5**	**3.0**
Males						
PND 22	1	54.32 ± 12.33	50.69 ± 15.12	50.98 ± 15.25	55.02 ± 16.38	57.48 ± 20.83
	2	60.04 ± 22.06	53.48 ± 18.79	49.87 ± 12.52	55.91 ± 18.26	58.65 ± 18.72
	3	53.49 ± 14.62	62.24 ± 17.58	57.20 ± 16.90	53.74 ± 17.45	62.32 ± 17.80
	4	55.06 ± 18.11	58.11 ± 16.90	54.95 ± 16.09	50.56 ± 16.87	53.91 ± 10.25
	5	53.26 ± 16.69	49.52 ± 18.41	47.87 ± 13.22	49.46 ± 19.29	52.16 ± 14.85
	Mean	**55.23**	**54.81 (-1%)**	**52.17 (-5.5%)**	**52.94 (-4%)**	**56.94 (+3.1%)**
PND 63±2	1	44.35 ± 7.16	42.72 ± 5.32	45.96 ± 9.21	40.90 ± 10.51	48.93 ± 8.35
	2	45.58 ± 9.41	45.56 ± 12.05	47.17 ± 11.41	49.78 ± 12.23	50.13 ± 13.83
	3	47.46 ± 11.97	50.28 ± 10.86	47.71 ± 10.31	54.92 ± 16.30	51.59 ± 13.24
	4	51.29 ± 14.68	49.71 ± 12.64	48.76 ± 9.06	58.36 ± 14.89	53.40 ± 16.87
	5	49.24 ± 9.14	46.53 ± 9.03	53.54 ± 13.87	54.00 ± 11.48	55.93 ± 11.08
	Mean	**47.58**	**46.96 (-13%)**	**48.62 (+2.2%)**	**51.6 (+8.4%)**	**51.0 (+7.2%)**
Females						
PND 22	1	52.21 ± 14.00	49.71 ± 12.49	55.04 ± 18.21	58.43 ± 16.09	49.83 ± 13.78
	2	53.23 ± 19.84	47.65± 13.13	56.16 ± 15.88	53.04 ± 15.56	47.06 ± 14.67
	3	53.26 ± 18.60	46.98 ± 13.74	54.65 ± 19.59	61.87 ± 21.74	50.80 ± 17.99
	4	52.84 ± 16.16	50.18 ± 14.86	56.63 ± 20.09	60.24 ± 18.66	51.12 ± 12.92
	5	54.74 ± 15.98	50.41 ± 12.38	52.24 ± 18.46	58.55 ± 17.42	50.92 ± 20.46
	Mean	**53.26**	**49.0 (-8%)**	**54.94 (+3.2%)**	**58.43 (+7.8%)**	**49.95 (-6.2%)**
PND 63±2	1	55.78 ± 14.99	54.88 ± 11.51	53.24 ± 12.43	62.71 ± 13.76	50.44 ± 9.58
	2	62.30 ± 18.61	55.71 ± 12.23	55.81 ± 14.88	55.68 ± 13.39	59.21 ± 16.59
	3	61.71 ± 15.95	57.32 ± 13.14	56.39 ± 12.6	66.47 ± 16.32	55.15 ± 13.22
	4	62.74 ± 17.68	59.72 ± 21.40	59.97 ± 14.83	61.44 ± 13.53	66.13 ± 17.00
	5	65.10 ± 20.91	59.89 ± 14.89	63.38 ± 16.68	66.41 ± 16.32	61.69 ± 15.55
	Mean	**61.53**	**57.47 (-6.6%)**	**57.76 (-6.1%)**	**62.54 (+1.6%)**	**58.52 (-4.9%)**

[a] Data were obtained from Tables F2 and F3, pages 575 - 578 in MRID 46749002
N= 16-20

d. **Learning and memory testing:** Passive avoidance in the offspring is presented in Table 12. Comparable findings in trials to criterion, latency and failure to learn were observed in both the males and females in the controls and treated groups. The only statistically significant ($p \leq 0.05$) finding was an increase in latency in females in the 1.0 mg/kg/day group in Session Two; but it is not a concern since the effect was not observed in a dose-related manner and other parameters are not affected..

TABLE 12. Passive avoidance performance in offspring (mean ± S.D.) [a]

Test day	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Males					
Session One:					
Number of rats tested	17	14	19	18	18
Trials to criterion	6.0 ± 3.0	5.8 ± 2.5	5.4 ± 2.6	4.7 ± 1.5	5.5 ± 2.4
Latency trial 1 (sec)	8.9 ± 7.0	10.8 ± 8.4	10.0 ± 7.5	5.9 ± 3.7	7.5 ± 5.0
Latency trial 2 (sec)	23.4 ± 18.4	19.2 ± 19.8	24.9 ± 18.9	26.3 ± 18.8	18.7 ± 15.6
Failed to learn (%)	1 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Session Two:					
Number of rats tested	16	14	19	18	17
Trials to criterion	3.6 ± 1.3	3.3 ± 1.3	3.4 ± 0.9	3.3 ± 1.1	3.2 ± 1.0
Latency trial 1 (sec)	24.0 ± 21.6	25.1 ± 24.0	25.6 ± 22.8	29.5 ± 25.6	22.4 ± 19.7
Females					
Session One:					
Number of rats tested	18	19	18	18	17
Trials to criterion	5.4 ± 1.9	4.6 ± 1.0	5.0 ± 2.7	4.6 ± 1.6	5.0 ± 2.6
Latency trial 1 (sec)	7.2 ± 4.6	7.1 ± 4.8	10.8 ± 14.2	6.7 ± 4.3	6.7 ± 3.8
Latency trial 2 (sec)	27.3 ± 23.2	27.6 ± 21.1	33.8 ± 19.0	30.6 ± 18.1	28.9 ± 20.0
Failed to learn (%)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	0 (0.0)
Session Two:					
Number of rats tested	18	19	17	18	17
Trials to criterion	3.2 ± 1.4	3.1 ± 1.4	3.0 ± 0.9	2.8 ± 0.8	2.9 ± 0.4
Latency trial 1 (sec)	30.2 ± 25.0	38.7 ± 21.6	48.9* ± 19.1	31.9 ± 23.8	26.0 ± 16.4

[a] Data obtained from Table E1 on page 483 of MRID 46749002.
* Statistically different from control, p≤0.05

Water maze performance is presented in Table 13 and does not indicate any treatment-related effects in either the male or female offspring. Males in the 3.0 mg/kg/day group in the learning session (session 1) had a statistically significant ($p \leq 0.05$) increase in the trials to criterion but all other parameters were comparable to controls and this number was still within the normal range for the historical control data of the laboratory. Twelve of 18 males in the 3 mg/kg/day dose required more than 10 trials to reach criterian compared to 4 of 17 males in controls. Further latency period was also increased in relation to controls. Thirteen of 18 high-dose males had latency period of 10-60 sec vs 8 of 17 males in controls which required 13-20 sec. In females in learning session 1, the trial to reach criterion increased significantly ($p < 0.05$) at 1.0 and 1.5 mg/kg/day groups but all other parameters were comparable to controls.

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TABLE 13. Water Maze Performance in Offspring (mean ± S.D.) [a]					
Test day	Dose (mg/kg/day)				
	Control	0.5	1.0	1.5	3.0
Males					
Session One:					
Number of rats tested	17	14	19	18	18
Trials to criterion	8.2 ± 2.4	9.0 ± 3.1	8.3 ± 2.6	7.8 ± 2.1	10.4* ± 2.7
Errors per trial	0.36 ± 0.22	0.36 ± 0.24	0.37 ± 0.17	0.33 ± 0.17	0.45 ± 0.23
Latency trial 2 (sec)	12.4 ± 6.4	10.8 ± 5.6	12.3 ± 8.0	11.6 ± 6.3	19.4 ± 17.0
Failed to learn (%)	1 (5.9)	1 (7.1)	1 (5.3)	0 (0.0)	1 (5.6)
Session Two:					
Number of rats tested	16	13	18	18	17
Trials to criterion	6.1 ± 1.7	6.8 ± 2.0	6.7 ± 2.5	6.3 ± 2.0	7.4 ± 3.4
Errors per trial	0.09 ± 0.11	0.13 ± 0.16	0.14 ± 0.14	0.11 ± 0.14	0.14 ± 0.16
Latency trial 1 (sec)	11.0 ± 4.6	9.5 ± 7.8	9.8 ± 6.6	11.3 ± 12.4	10.0 ± 8.4
Females					
Session One:					
Number of rats tested	18	19	18	18	18
Trials to criterion	7.0 ± 1.8	8.7 ± 2.8	8.9* ± 2.2	8.9* ± 2.0	8.9 ± 2.8
Errors per trial	0.32 ± 0.19	0.42 ± 0.31	0.36 ± 0.17	0.35 ± 0.12	0.37 ± 0.16
Latency trial 2 (sec)	10.8 ± 4.4	14.6 ± 8.8	13.4 ± 7.2	12.9 ± 5.2	13.2 ± 9.1
Failed to learn (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Session Two:					
Number of rats tested	18	19	18	18	17
Trials to criterion	7.8 ± 3.3	6.7 ± 1.9	7.9 ± 3.2	7.9 ± 3.2	6.9 ± 2.8
Errors per trial	0.16 ± 0.14	0.20 ± 0.17	0.21 ± 0.21	0.19 ± 0.12	0.10 ± 0.12
Latency trial 1 (sec)	11.2 ± 7.2	13.8 ± 9.0	13.8 ± 9.4	12.3 ± 7.7	9.8 ± 4.9

[a] Data obtained Table E2 on page 484 in MRID 46749002.
* Statistically different from control, $p \leq 0.05$

e. **Hematology:** No treatment-related findings were identified in the hematology results for the offspring.

5. **Postmortem results:**

a. **Brain weight:** Mean brain weight data are presented in Table 14. There were no treatment-related differences in terminal body weight, brain weight or brain-to-body weight ratio in any of the rats.

TABLE 14. Mean (±SD) brain weight data [a]					
Parameters measured	***Dose (mg/kg/day)***				
	Control	**0.5**	**1.0**	**1.5**	**3.0**
Males					
Day 21 postpartum (Subset 1)					
Number of animals	10	9	9	10	10
Terminal body weight (g)	44.4 ± 7.2	49.2 ± 6.0	51.6 ± 3.6	48.1 ± 6.9	44.8 ± 9.1
Brain weight (g)	1.65 ± 0.14	1.65 ± 0.09	1.72 ± 0.10	1.70 ± 0.11	1.63 ± 0.17
Brain-to-body weight ratio[b]	3.78 ± 0.43	3.38 ± 0.35	3.35 ± 0.24	3.59 ± 0.45	3.73 ± 0.47
Day 69 postpartum (Subset 4)					
Number of animals	10	10	10	10	10
Terminal body weight (g)	396.0 ± 36.5	413.1 ± 51.8	432.8 ± 49.6	414.3 ± 33.2	428.5 ± 27.5
Brain weight (g)	2.23 ± 0.11	2.26 ± 0.24	2.32 ± 0.15	2.33 ± 0.10	2.31 ± 0.13
Brain-to-body weight ratio	0.57 ± 0.05	0.55 ± 0.04	0.54 ± 0.07	0.56 ± 0.04	0.54 ± 0.03
Females					
Day 21 postpartum (Subset 1)					
Number of animals	10	10	10	10	9
Terminal body weight (g)	47.1 ± 4.5	45.3 ± 8.8	46.2 ± 7.5	50.3 ± 4.4	47.5 ± 8.0
Brain weight (g)	1.67 ± 0.06	1.55 ± 0.17	1.59 ± 0.15	1.66 ± 0.11	1.62 ± 0.09
Brain-to-body weight ratio	3.48 ± 0.24	3.50 ± 0.47	3.50 ± 0.42	3.32 ± 0.20	3.47 ± 0.43
Day 69 postpartum (Subset 4)					
Number of animals	10	10	10	10	10
Terminal body weight (g)	235.4 ± 24.8	234.2 ± 27.5	240.9 ± 21.3	237.8 ± 27.5	236.8 ± 33.5
Brain weight (g)	2.13 ± 0.18	2.06 ± 0.16	2.05 ± 0.16	2.11 ± 0.12	2.06 ± 0.15
Brain-to-body weight ratio	0.91 ± 0.07	0.89 ± 0.09	0.85 ± 0.06	0.90 ± 0.11	0.88 ± 0.13

[a] Data from pages 476-477 and 700-701in MRID 46749002.
[b] Ratio (%) = brain weight/ terminal weight x 100

b. Neuropathology:

1. **Macroscopic examination:** No treatment-related effects were observed on gross examination.

2. **Microscopic examination:** Histopathologic examinations were taken on the rats at two endpoints, PND 22 in juveniles and PND 70 in adults, in the control and 3.0 mg/kg/day groups. There were no treatment-related effects seen at the histopathological examination. The only finding observed in the juveniles was a mild hydrocephalus in one of the control females; not a treatment-related finding. Findings in the adults rats such as focal pyramidal layer defect of hippocampus, focal minerlization of thalamus, axon degeneration within the trapezoid body of the brain stem, neurofibril aggregation within ganglionic neurons in the gasserian ganglia, nerve fiber degeneration (peripheral and spinal nerve roots), hydrocephalus, retinal dysplasia and myositis were either typical of findings in adult rats, identified in both controls and treated rats or observed in only minimal numbers (1-2) making them not toxicologically significant.

   Morphometric evaluation, presented in Table 15 revealed no treatment-related findings in the gross or micrometric linear brain measurements taken on the juveniles (PND 22) or the adults (PND 70). The gross measurements were taken on all dose groups; however, the micrometer measurements were only performed on the control and 3.0 mg/kg/day groups.

TABLE 15. Mean (±SD) morphometric data [a]					
Parameters measured	**Dose (mg/kg/day)**				
	Control	**0.5**	**1.0**	**1.5**	**3.0**
Males					
Day 22 postpartum (Subset 1)					
AP of cerebrum (mm)[b]	14.17 ± 0.517	14.28 ± 0.254	14.42 ± 0.295	14.40 ± 0.414	14.14 ± 0.497
AP of cerebellum (mm)[b]	7.19 ± 0.491	7.02 ± 0.291	7.09 ± 0.203	7.33 ± 0.271	7.14 ± 0.392
Frontal cortex (μ)[c]	1862.7 ± 80.67	NR	NR	NR	1885.8 ± 114.31
Parietal cortex (μ)[c]	1824.6 ± 99.95	NR	NR	NR	1823.1 ± 85.22
Striatum (caudate-putamen) [c](μ)	2824.8 ± 116.06	NR	NR	NR	2815.8 ± 143.40
Corpus callosum (μ)[c]	217.2 ± 35.76	NR	NR	NR	225.6 ± 35.37
Hippocampus (μ)[c]	1307.7 ± 80.16	NR	NR	NR	1326.9 ± 88.73
Cerebellum (μ)[c]	5037.0 ± 317.63	NR	NR	NR	4995.0 ± 361.18
Day 70 postpartum (Subset 4)					
AP of cerebrum (mm)[b]	16.04 ± 0.295	16.03 ± 0.306	16.13 ± 0.226	16.13 ± 0.216	16.02 ± 0.169
AP of cerebellum (mm)[b]	7.73 ± 0.527	7.89 ± 0.223	7.83 ± 0.467	8.08 ± 0.193	7.54 ± 0.295
Frontal cortex (μ)[c]	1918.5 ± 112.79	NR	NR	NR	1905 ± 87.75
Parietal cortex (μ)[c]	1923 ± 95.60	NR	NR	NR	1954.5 ± 90.29
Striatum (caudate-putamen) [c](μ)	3259.2 ± 111.89	NR	NR	NR	3261.6 ± 83.48
Corpus callosum (μ)[c]	271.2 ± 50.26	NR	NR	NR	266.4 ± 26.86
Hippocampus (μ)[c]	1483.5 ± 109.19	NR	NR	NR	1500 ± 104.40
Cerebellum (μ)[c]	5868 ± 276.84	NR	NR	NR	5814 ± 326.74
Females					
Day 22 postpartum (Subset 1)					
AP of cerebrum (mm)[b]	14.26 ± 0.310	13.93 ± 0.618	14.12 ± 0.533	14.28 ± 0.175	14.08 ± 0.311
AP of cerebellum (mm)[b]	7.00 ± 0.435	6.92 ± 0.541	7.10 ± 0.245	7.27 ± 0.316	7.28 ± 0.291
Frontal cortex (μ)[c]	1814.1 ± 78.79	NR	NR	NR	1830.3 ± 106.76
Parietal cortex (μ)[c]	1815.9 ± 67.90	NR	NR	NR	1832.3 ± 75.68
Striatum (caudate-putamen) [c](μ)	2817.2 ± 149.22	NR	NR	NR	2801.1 ± 109.75
Corpus callosum (μ)[c]	213.4 ± 28.89	NR	NR	NR	236.9 ± 35.20
Hippocampus (μ)[c]	1326.0 ± 67.05	NR	NR	NR	1318.3 ± 88.17
Cerebellum (μ)[c]	4943.0 ± 144.48	NR	NR	NR	4860.0 ± 143.09

TABLE 15. Mean (±SD) morphometric data [a]					
Parameters measured	**Dose (mg/kg/day)**				
	Control	**0.5**	**1.0**	**1.5**	**3.0**
Day 70 postpartum (Subset 4)					
AP of cerebrum (mm)[b]	15.57 ± 0.457	15.50 ± 0.362	15.57 ± 0.50	15.80 ± 0.287	15.44 ± 0.288
AP of cerebellum (mm)[b]	7.58 ± 0.535	7.73 ± 0.495	7.38 ± 0.514	7.76 ± 0.521	7.76 ± 0.438
Frontal cortex (µ)[c]	1903.5 ± 88.16	NR	NR	NR	1911 ± 74.23
Parietal cortex (µ)[c]	1907.3 ± 74.20	NR	NR	NR	1913.3 ± 59.88
Striatum (caudate-putamen)[c] (µ)	3186 ± 192.44	NR	NR	NR	3117.6 ± 146.73
Corpus callosum (µ)[c]	274.8 ± 42.31	NR	NR	NR	267 ± 62.24
Hippocampus (µ)[c]	1458.8 ± 61.35	NR	NR	NR	1418.3 ± 75.78
Cerebellum (µ)[c]	5637 ± 373.75	NR	NR	NR	5478 ± 280.07

[a] Data obtained from Tables 5-12, pages 916-923 in MRID 46749002.
[b] gross linear measurements; AP = anterior to posterior
[c] micrometric linear brain measurements
NR = not recorded

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigator concluded that the male and female F_1 rat offspring were less sensitive to the effects of DPX-KN128 (Indoxacarb) than the dams. Maternal animals showed a statistically significant decrease in body weight gain and decreased food consumption in the highest dose group at 3.0 mg/kg/day. There was mortality of four rats in the 3.0 mg/kg/day group with 3/4 considered treatment-related because of accompanying signs of neurotoxicity. The signs observed included: chromorhinorrhea, dehydration, decreased motor activity, lost righting reflex, lacrimation, rales, and ataxia. While there was increased pup mortality in the 3.0 mg/kg/day group, the pups were from the dams with increased clinical signs and mortality, indicating effects secondary to maternal toxicity. The investigator identified no increase in any of the clinical observations or abnormal autonomic function in the offspring. There was an increase in body weight gain and food consumption in the 3.0 mg/kg/day male offspring during the post-dosing period that was not observed in the female offspring. No adverse effects were observed on any offspring in any of the neurological parameters measured including all of the testing and histopathological parameters.

The maternal no-observable-adverse-effect level (NOAEL) for DPX-KN128 (Indoxacarb) was 1.5 mg/kg/day based on transient and minimal maternal effects observed in the 1.5 mg/kg/day dose group, and on mortality, decreases in body weight gain and reduction in food consumption observed in the 3.0 mg/kg/day dose group.

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The developmental no-observable-adverse-effect level (NOAEL) for DPX-KN128 (Indoxacarb) was 1.5 mg/kg/day for the F_1 generation male rats and 3.0 mg/kg/day for the F_1 generation female rats. There was an increase in body weight gain and food consumption during the post-dosing period for the 3.0 mg/kg/day males. There were no accompanying effects on motor activity, learning and memory, neuromorphometry or neurohistopathology. The investigator concluded that DPX-KN128 is not a developmental neurotoxicant.

B. REVIEWER COMMENTS:

In the maternal animals, a total of 0, 1, 1, 0, and 4 rats were found dead or sacrificed in moribund condition in the 0, 0.5, 1, 1.5 or 3.0 mg/kg/day dose groups, respectively. Three of the four deaths in the 3.0 mg/kg/day group were considered treatment-related because of accompanying toxicological signs while the others were either dosing errors or incidental deaths. The severity of the clinical observations was not included in the study report. No treatment-related findings at necropsy were identified for rats that died or were sacrificed; although those dying from dosing errors did have a perforated esophagus.

Treatment-related decreases in mean body weight, body weight gain and food consumption in the 3.0 mg/kg/day dams were observed primarily during gestation, compared to controls. A statistically significant decrease ($p \leq 0.01$) in mean body weight was observed on GD 13, 6 and 8% in the 1.5 and 3.0 mg/kg/day groups, respectively, compared to controls. The mean body weight gain in the 3.0 mg/kg/day dams, however, was decreased statistically ($p \leq 0.01$) and toxicologically (23%) on GDs 6-20. A corresponding decrease in food consumption was observed in the 1.5 (12%) and 3.0 (19%) mg/kg/day females on GDs 9-12 compared to controls and decreases of 7 and 17% compared to controls, respectively, during GDs 6-20. Mean body weight was decreased statistically in the 1.5 and 3.0 mg/kg/day dams on LDs 0 and 4 but by less than 10% compared to controls. Overall, during gestation, the 3.0 mg/kg/day dams demonstrated a dose dependent decrease in mean body weight, body weight gain and food consumption although it was not always statistically significant. The dams were more comparable to controls at all dose levels throughout the lactation period except for early lactation as mentioned above.

All dams that survived gestation delivered litters with a very similar gestation duration. There was an increase in the number of stillborn pups in the 3.0 mg/kg/day group (14) compared to controls (1). The number of pups that died during the first four days was also statistically significantly increased ($p \leq 0.01$) in the 3.0 mg/kg/day group (10.7%) compared to the controls (1%).

Maternal animals were affected at the highest dose administered, 3.0 mg/kg/day. This was seen as increased mortality, clinical signs, decreased body weight gain and decreased food consumption.

The maternal systemic and neurotoxicity LOAEL for DPX-KN 128 (Indoxacarb) in rats is 3.0 mg/kg/day, based on adverse clinical signs, decreased body weight gain and decreased food consumption. The maternal NOAEL for DPX-KN 128 (Indoxacarb) is 1.5 mg/kg/day.

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In the offspring, treatment-related effects were seen on pup survival and in increased pup mortality during the first 4 days of life as stated above. There was an increase in the number of stillborn pups in the 3.0 mg/kg/day group (14) compared to controls (1). The number of pups that died during the first four days was also a statistically significant increase ($p \leq 0.01$) in the 3.0 mg/kg/day group (10.7%) compared to the controls (1%).

The only treatment-related effect on body weight was observed at birth in the 3.0 mg/kg/day group. After this date, there were no effects on clinical signs, body weight changes or food consumption. While the reviewer agrees with the investigator that there were some statistically significant increases in body weight gain and food consumption in the male offspring in the highest dose group, 3.0 mg/kg/day, the increases were less than 10%, sporadic and not considered toxicologically significant. No effects on these parameters were observed in any of the females.

There was no effect observed on any of the offspring in sexual maturation. FOB parameters, motor activity,acoustic startle results, and learning and memory testing were all comparable between the control and treated animals. Mean brain weight and the brain-to-body weight ratio were also comparable between the treated and non-treated animals. No treatment-related effects were observed at the gross or microscopic examination of the nervous systems or on the morphometric measurements of various portions of the brain.

The offspring systemic and neurotoxicity LOAEL for DPX-KN 128 (Indoxacarb) in rats is 3.0 mg/kg/day based on the increased number of stillbirths, decreased pup body weight at birth and increased pup mortality during PND 1-4. The offspring NOAEL for DPX-KN 128 (Indoxacarb) is 1.5 mg/kg/day.

C. **STUDY DEFICIENCIES:** Study authors provided acceptable additional details on the conduct of the study. Study is upgraded to acceptable/non-Guideline. See attachments

APPENDIX A: HISTORICAL DATA FOR WATERMAZE TESTING

Historical Watermaze Data

The following table presents the summary of historical data of watermaze testing in male and female Crl:CDBR VAF/Plus rats in studies dated from 2-2-94 until 12-2-05, provided by the performing laboratory. The rats were administered test substance by the following methods: gavage, intravenous, subcutaneous, intramuscular, drinking water, diet and percutaneous application.

TABLE 1. Mean historical data on watermaze testing[a]

Parameter	Average	Minimum	Maximum	# of studies included
Males				
Learning session (Day 1) No. of animals	21.5	10	32	87
Total trials to criterion	8.8	7.3	10.7	87
Errors per trial	0.4	0.3	0.6	87
Latency trial 2	14.7	8.8	24.6	87
Failed to learn (%)	0.7 (3)	0 (0.0)	3 (13.6)	87
Retention session (Day 2) No. of animals	20.8	10	31	87
Total trials to criterion	6.4	5.2	9.9	87
Errors per trial	0.1	0.0	0.5	87
Latency trial 1	9.6	5.4	23.5	87
Females				
Learning session (Day 1) No. of animals	21.5	10	33	87
Total trials to criterion	8.9	7.1	11.2	87
Errors per trial	0.4	0.2	0.5	87
Latency trial 2	14.3	9.5	20.1	87
Failed to learn (%)	0.6 (2.8)	0 (0.0)	3 (15.8)	87
Retention session (Day 2) No. of animals	20.8	10	30	87
Total trials to criterion	7.0	5.4	9.3	87
Errors per trial	0.2	0.1	0.5	87
Latency trial 1	11.1	6.5	21.9	87

[a] Data provided for from MRID 46749002, Appendix R, pages 1069 and 1092.

APPENDIX B

Statistical Methodology

1.2.1 Within Session

Each analysis endpoint was analyzed, by posttreatment session, with a repeated measure analysis of variance (RANOVA). Factors in the model included treatment group (TRT), SEX, time interval or block (TIME), and the following interaction terms: TRT*SEX, TRT*TIME and TRT*SEX*TIME. The SAS® procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by evaluating Akaike's Information Criterion (AIC) for compound symmetry (CS) and first-order autoregressive [AR(1)] structures.

Interaction terms that included TRT*SEX (TRT*SEX and TRT*SEX*TIME) were evaluated at the 0.01 significance level. If any of these interaction terms were significant the analysis was conducted separately for each sex with a RANOVA including the following terms: TRT, TIME, and TRT*TIME.

In the final model, monotonicity of dose response was examined using sequential trend tests based on ordicanl spacing of dose levels. Dose response trend tests on treatment means were preceded by two linear treatment by time interaction tests: 1) linear trend in treatment by linear trend in time (LinTRT*LinTIME); and 2) linear trend in treatment by quadratic trend in time (LinTRT*QdrTIME). Within the framework of the RANOVA, dose response linear trend tests were performed at the 0.05 significance level. If either of the two interactions was significant at the 0.05 significance level the trend tests were performed for each time interval and across the pooled time intervals. If neither interaction was significant the trend tests were performed across the pooled time intervals only.

Nonmonotonic dose responses were evaluated whenever no significant dose-response linear trends were detected but TRT and/or TRT*TIME interaction was significant at the 0.01 level. Within the framework of the RANOVA, pairwise comparisons were made for each individual treated group with the control group at the 0.01 significance level. If TRT*TIME was significant, the comparisons were conducted for each time interval and over the entire sesion. If only the TRT effect was significant, the comparisons were conducted across the entire session only.

1.2.2 Across Sessions

For each analysis endpoint, the total value for each session was calculated. Each analysis endpoint was then analyzed with a repeated measure analysis of variance (RANOVA).

Factors in the model included treatment group (TRT), SEX, SESSION; and the following interaction terms: TRT*SEX, TRT*SESSION and TRT*SEX*SESSION. The SAS® procedure PROC MIXED was used for analysis with the random effect of animal included a the repeated measurement. The covariance structure across time was selected by evaluating Akaike's Information Criterion (AIC) for compound symmetry (CS) and first-order autoregressive [AR(1)] structures.

Interaction terms that included TRT*SEX (TRT*SEX and TRT*SEX*SESSION) were evaluated at the 0.01 significance level. If any of these interaction terms were significant th analysis was conducted separately for each sex with a RANOVA including the following terms: TRT, SESSION, and TRT*SESSION.

In the final model, monotonicity of dose response was examined using sequential trend tests based on ordicanl spacing of dose levels. Dose response trend tests on treatment means wer preceded by the following linear treatment by session interaction tests: 1) linear trend in treatment by linear trend in session (LinTRT*LinSESSION); and, for motor activity, 2) line trend in treatment by quadratic trend in session (LinTRT*QdrSESSION). (Note that the latt interaction term was not evaluated for startle response because there were only two sessions and therefore the quadratic term in sessions does not exist.) Within the framework of the RANOVA, dose response linear trend tests were performed at the 0.05 significance level. I either of the two interactions was significant at the 0.05 significance level the trend tests we performed for each session and across the pooled sessions. If neither interaction was significant the trend tests were performed across the pooled sessions only.

Nonmonotonic dose responses were evaluated whenever no significant dose-response linear trends were detected but TRT and/or TRT*SESSION interaction was significant at the 0.01 level. Within the framework of the RANOVA, pairwise comparisons were made for each individual treated group with the control group at the 0.01 significance level. If TRT*SESSIO was significant, the comparisons were conducted for each session and across the pooled sessio If only the TRT effect was significant, the comparisons were conducted across the pooled sessions only.