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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

July 31, 2007
TXR # 0053421

SUBJECT: I.D. No.: 129098 Fluazinam: Review of the developmental neurotoxicity study (2005, MRIDs 46534401, 47018301, 47037001

PC Code: 129098
DP Barcode: D317746 (original) and D339160 (response)

FROM: J. D. Doherty, Ph.D. 7/31/07
ReRegistration Branch 3
Health Effects Division (7509P)

TO: Tony Kish
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And

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THROUGH: Richard Loranger R. Loranger
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CONCLUSIONS

The developmental neurotoxicity study (2005, MRIDs 46534401) with fluazinam was submitted and supported by the registrant's response to provide additional information (2006, MRID 47019301) including the range finding study (2005, MRID 47037001). The definitive study was determined to be Acceptable/Non-Guideline and may be used for regulatory purposes. A copy of the DER is attached. The study is further described together with the Executive Summary in the table attached.

Received 11/2/07 aw

Table. Study Reviewed

Study Identification	Executive Summary
83-6. (870.6300). Developmental Neurotoxicity – rats Huntingdon Document No.; ISK 272/042019, March 31, 2005.	In a developmental neurotoxicity study (2005, MRID 46534401), Fluazinam (97.8% a.i.; lot # A629/1995, impurity #5 0.09%) was administered by gavage to 24 Crl:CD® (SD) IGS BR rats/sex/dose at 0, 2, 10 or 50 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20. The pups were administered the same doses by gavage from postnatal days (PND) 7 to 20 or 21. Maternal evaluation consisted of a Functional Operational Battery (FOB) was performed on GDs 12 and 18, and on post partum days (PPDs) 35, 45 and 60. Additional behavioral assessments included: motor activity on GD 15 and PPD 60; auditory startle habituation on GD 19 and PPD 58; and learning and memory on LD 16 and PPD 61. From each maternal group, 12 were sacrificed on both LD 21 and PPD 66; of these, 10/group were selected for neuropathology procedures. The additional assessment of the dams is related to trying to further characterizing neurotoxicity that is attributed to impurity #5. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (watermaze testing), and neuropathology at days 23/24 and on PND day 66. On PND 21, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed. *Maternal parameters.* No effects on absolute body weight were noted. Mean body weight *gain* for GDs 6-14 was significantly decreased (14%) at 10 and 50 mg/kg/day and during GDs 6-20 at 50 mg/kg/day (10%). Mean body weight gain during lactation was not affected. Food consumption during gestation was comparable to controls but during lactation was significantly decreased at 10 (10 to 14%) and 50 mg/kg/day (7 to 11%). Mean body weight and body weight gain post weaning (days 28-63) were not affected. The weight *gain* and food consumption data that occurs in the absence of absolute weight differences for the dams are not considered to be of sufficient magnitude to be included as a true toxic response. Reproductive parameters and behavioral assessments, including FOB, motor activity, auditory startle reflex habituation and learning and memory, were not affected by treatment. No treatment-related changes were observed at either the necropsy on LD 21 or PPD 66. The characteristic grey matter vacuolation attributed to fluazinam in previous studies was not seen at LD 21 or PPD 66. **The maternal LOAEL for Fluazinam was not established. The maternal NOAEL is > 50 mg/kg/day.** The weight *gain* and food consumption data that occurs in the absence of absolute weight differences for the dams are not considered to be sufficient of sufficient magnitude to be included as a true toxic response. *Offspring parameters.* No treatment-related effects were observed on litter size at birth or survival to weaning. Birth weight was lower in females at 10 and 50 mg/kg/day (both 6%, $p < 0.01$) but not in males. Mean offspring body weight was significantly decreased in males and females at 10 (6-11%) and 50 mg/kg/day (6-16%) during lactation. Mean body weight gain was significantly decreased in male and female pups at 10 (4-24%) and 50 mg/kg/day (16-35%) during lactation. During the post-weaning period (PNDs 28-63), mean body weight was significantly decreased in males and females at 10 (3-7%) and 50 mg/kg/day (7-15%). However, post weaning body weight gain was essentially comparable to the control group. The mean age of completion of balano-preputial separation was significantly delayed at 10 and 50 mg/kg/day. Rearing counts in female pups were decreased on day 21 in the 10 (to a mean of 3.5 vs. 8.1 in the controls) and 50 (mean 3.7) mg/kg/day dose groups. Dark and/or distended abdomens observed in a total of 12 offspring from 4 litters at 50 mg/kg/day were considered treatment-related since similar signs were observed in the preliminary study. The peak amplitude in the auditory startle response was affected in males in the high dose group at day 23/24. Absolute brain weight for high dose males was 6.1% decreased on PND 21 and slight changes in brain width were noted. No treatment-related effects were observed on other behavioral assessments, including FOB, motor activity or learning and memory. Grey matter vacuolation was not seen in the LD21 neuropathology assessment. A single isolated incident of grey matter vacuolation in the high dose group at day 66 was not considered related to treatment. **The offspring LOAEL is 10 mg/kg/day based on decreased body weight and body weight gain and delay in completion of balano-preputial separation. The offspring NOAEL is 2 mg/kg/day.** This developmental neurotoxicity is classified as **Acceptable/Non-Guideline** and may be used for

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Study Identification	Executive Summary
	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.

Version date: 1/18/07

DATA EVALUATION RECORD

FLUAZINAM

OPPTS 870.6300

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY IN RATS
MRID 46534401

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 109-2005

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Disclaimer

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

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EPA Reviewer: John Doherty **Signature:** 7/31/07
Registration Action Branch 3, Health Effects Division (7509C) **Date**
EPA Work Assignment Manager: Pv Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date** P.V Shah. 7/31/07

TXR#: 0053421

DATA EVALUATION RECORD

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

PC CODE: 129098

DP BARCODE: D317746
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Fluazinam (97.8% a.i.)

SNYONYMS: B1216, IKF 1216 and PP192

CHEMICAL NAMES: 3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-%,%,%-trifluoro-2,6-dinitro-p-toluidine (IUPAC Chemical name); 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)-phenyl]-5-(trifluoromethyl)-2-pyridinamine (CA Chemical name)

CITATION: Fulcher, S.M.(2005) Technical Fluazinam: Developmental neurotoxicity study in the rat by oral (gavage) administration. Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England. Document Number ISK 272/042019, March 31, 2005. MRID 46534401. Unpublished.

-- (2006) Technical Fluazinam Developmental Neurotoxicity Study in the Rat by Oral (Gavage) Administration Response to Reviewers Comments" Signed December 28, 2006. MRID No.: 47018301.

Fulcher, S.M. (2005) Technical Fluazinam Preliminary Developmental Neurotoxicity Study in the Rat by Oral Gavage Administration to CD Rats and Their Offspring. Huntingdon Life Sciences, Ltd. Study No.: ISK 271/040029, November 3, 2005. MRID No>: 47037001. .

SPONSOR: Ishihara Sangyo Kaisha, Ltd., Osaka, Japan

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2005, MRID 46534401), Fluazinam (97.8% a.i.; lot # A629/1995, impurity #5 0.09%) was administered by gavage to 24 Crl:CD® (SD) IGS BR rats/sex/dose at 0, 2, 10 or 50 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20. The pups were administered the same doses by gavage from postnatal days (PND) 7 to 20 or 21. Maternal evaluation consisted of a Functional Operational Battery (FOB) was

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performed on GDs 12 and 18, and on post partum days (PPDs) 35, 45 and 60. Additional behavioral assessments included: motor activity on GD 15 and PPD 60; auditory startle habituation on GD 19 and PPD 58; and learning and memory on LD 16 and PPD 61. From each maternal group, 12 were sacrificed on both LD 21 and PPD 66; of these, 10/group were selected for neuropathology procedures. The additional assessment of the dams is related to trying to further characterizing neurotoxicity that is attributed to impurity #5. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (watermaze testing), and neuropathology at days 23/24 and on PND day 66. On PND 21, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

Maternal parameters. No effects on absolute body weight were noted. Mean body weight *gain* for GDs 6-14 was significantly decreased (14%) at 10 and 50 mg/kg/day and during GDs 6-20 at 50 mg/kg/day (10%). Mean body weight gain during lactation was not affected. Food consumption during gestation was comparable to controls but during lactation was significantly decreased at 10 (10 to 14%) and 50 mg/kg/day (7 to 11%). Mean body weight and body weight *gain* post weaning (days 28-63) were not affected. The weight *gain* and food consumption data that occurs in the absence of absolute weight differences for the dams are not considered to be of sufficient magnitude to be included as a true toxic response. Reproductive parameters and behavioral assessments, including FOB, motor activity, auditory startle reflex habituation and learning and memory, were not affected by treatment. No treatment-related changes were observed at either the necropsy on LD 21 or PPD 66. The characteristic grey matter vacuolation attributed to fluazinam in previous studies was not seen at LD 21 or PPD 66. **The maternal LOAEL for Fluazinam was not established. The maternal NOAEL is > 50 mg/kg/day.** The weight *gain* and food consumption data that occurs in the absence of absolute weight differences for the dams are not considered to be sufficient of sufficient magnitude to be included as a true toxic response.

Offspring parameters. No treatment-related effects were observed on litter size at birth or survival to weaning. Birth weight was lower in females at 10 and 50 mg/kg/day (both 6%, $p < 0.01$) but not in males. Mean offspring body weight was significantly decreased in males and females at 10 (6-11%) and 50 mg/kg/day (6-16%) during lactation. Mean body weight gain was significantly decreased in male and female pups at 10 (4-24%) and 50 mg/kg/day (16-35%) during lactation. During the post-weaning period (PNDs 28-63), mean body weight was significantly decreased in males and females at 10 (3-7%) and 50 mg/kg/day (7-15%). However, post weaning body weight gain was essentially comparable to the control group. The mean age of completion of balano-preputial separation was significantly delayed at 10 and 50 mg/kg/day. Rearing counts in female pups were decreased on day 21 in the 10 (to a mean of 3.5 vs. 8.1 in the controls) and 50 (mean 3.7) mg/kg/day dose groups. Dark and/or distended abdomens observed in a total of 12 offspring from 4 litters at 50 mg/kg/day were considered treatment-related since similar signs were observed in the preliminary study. The peak amplitude in the auditory startle response was affected in males in the high dose group at day 23/24. Absolute brain weight for high dose males was 6.1% decreased on PND 21 and slight changes in brain width were noted. No treatment-related effects were observed on other behavioral assessments, including FOB, motor activity or learning and memory. Grey matter vacuolation was not seen in the LD21 neuropathology assessment. A single isolated incident of grey matter vacuolation in the high dose group at day 66 was not considered related to treatment. **The offspring LOAEL is 10 mg/kg/day based on decreased body weight and body weight gain and delay in completion of balano-preputial separation. The offspring NOAEL is 2 mg/kg/day.**

This developmental neurotoxicity is classified as **Acceptable/Non-Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental

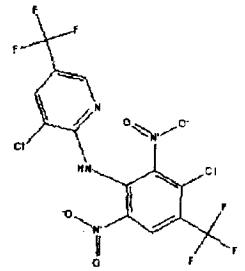
This developmental neurotoxicity is classified as **Acceptable/Non-Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6): OECD 426 (draft) due to the pending review of the positive control data.
The DNT Work Group determined that this study can be classified as Acceptable. This study does not satisfy the guideline requirements for a developmental neurotoxicity study and is classified as NonGuideline pending review of all available positive control data. J. Rowland, 07/31/2007.]

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Fluazinam

Description:	yellow powder
Lot #:	A629/1995
Purity:	97.8% a.i. (level of impurity #5 = 0.09%)
CAS # of TGAI:	79622-59-6
Stability:	Expiry date - July 2005
CAS No.	79622-59-6
Structure:	

2. **Vehicle:** 0.5% Sodium Carboxymethylcellulose in reverse osmosis water

3. **Test animals:**

Species:	Rat	
Strain:	Crl:CD7(SD) IGS BR	
Age at cohousing:	Females: 12-13 weeks	
Source:	Charles River (UK) Ltd., Margate, Kent, England	
Housing:	Stainless steel cages: up to 4 females during acclimation; dams and litters during LD 17 until weaning; litters from weaning until PND 28; up to 4 littermates after PND 28 Polypropylene cages: 1 male and 1female during mating; dam and litter GD 17 to LD 17	
Diet:	SDS VRF1 Certified Diet (Special Diet Services, Witham, Essex, England) *ad libitum*	
Water:	Municipal tap water *ad libitum.*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	Filtered fresh air that was not recirculated

1. **In life dates**: Start: January 14, 2004; End: November 18, 2004

2. **Study schedule**: Mated female CD rats (24/dose group) were administered the test material by gavage from gestation day (GD) 6 through lactation day (LD) 20. On postnatal day (PND) 4, litters were standardized to 8 pups, sexes were represented as equally as possible. Pups were treated from PND 7 to PND 20 or 21. Dams were perfused on Days 21 and 66 post partum and offspring were perfused on Days 21 and 66 of age for neuropathological assessment.

3. **Mating procedure**: The females were supplied as four littermates from each of 29 litters. The littermates were paired on the same day with stock males on a 1:1 basis over a 15-day period (the spread of mating was necessary in order to control the number of offspring having behavioral tests performed on any one day). Animals were allocated to study on GD 0 when positive evidence of mating was detected. *Only females with sperm in a vaginal smear or at least three copulation plugs were selected.*

4. **Animal assignment**: Females with positive evidence of mating were allocated to group and cage position in sequence of mating, as shown in Table 1.

TABLE 1. Study design

Experimental parameter	Dose (mg/kg/day)			
	0	2 (1.3 to 1.74)#	10 (8.5 to 9.3)#	50
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	24	24	24	24
FOB (GDs 12 and 18)	12	12	12	12
FOB (LDs 6 and 18, PPDs 35, 45, and 60)	10	10	10	10
Motor activity (GD 15, PPD 60)	10	10	10	10
Auditory startle habituation (GD 19, PPD 58)	10	10	10	10
Learning and memory (LD 16, PPD 61)	10	10	10	10
Brain weight, neuropathology, morphometrics (LD 21, PPD 66)	10	10	10	10
Offspring				
	Minimum No. of offspring assigned[a]			
FOB (PNDs 4, 11, 21, 35, 45 and 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PNDs 13, 17, 22 and 60)	10/sex	10/sex	10/sex	10/sex
Acoustic startle habituation and pre-pulse inhibition (PND 23/24 and 58)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PND 23/24 and 61)	10/sex	10/sex	10/sex	10/sex
Brain weight, neuropathology, morphometrics (PNDs 21 and 66)	10/sex	10/sex	10/sex	10/sex

[a] One male and/or female per litter with a minimum of 10/sex were assigned.
GD = Gestation Day; LD = Lactation Day; PPD = Post Partum Day; PND = Postnatal Day.

#numbers in () are range of doses based on analytical value.

5. **Dose selection rationale:** In a preliminary developmental neurotoxicity study (HLS Report number ISK271/040029, MRID No.: 47037001), mortality was observed in directly dosed offspring at dosages of 100 and 200 mg/kg/day. At 50 mg/kg/day, there were effects on offspring body weight. A copy of the study was obtained from the registrant and additional details of the effects seen at 50, 100 and 200 mg/kg/day in both the dams and pups were noted to justify the selection of 50 mg/kg/day as an appropriate high dose for the definitive study.

6. **Dosage administration:** Parental females were dosed from GD 6 to LD 20 or 21 by gavage using a graduated syringe and a rubber catheter at a dose volume of 5 ml/kg body weight. Offspring were dosed from PND 7 to PND 20 or 21 by gavage using a 1 mL syringe and an 18 gauge flexible plastic cannula.

7. **Dosage preparation and analysis:** The technical chemical was ground using a pestle and mortar and small amount of vehicle. The mixture was then ground again, ensuring a thorough mixing of the test article and vehicle. The suspension was transferred to an appropriate container and the remaining vehicle added to prepare the proper concentration. Formulations were prepared fresh daily Monday to Friday, with weekend formulations prepared on the preceding Friday and stored at 4EC until the day of use.

   Before dosing began, homogeneity and stability of the 0.4 and 10 mg/mL were tested on samples from the top, middle, and bottom of the bottle of formulations stored for up to three days at ambient temperature. Results of the initial assessments were unacceptable; therefore, the dosing was delayed while a second trial was performed. Results from the second trial and confirmation by a third trial were acceptable. Samples of each formulation for use on the first day of treatment of parental females and during the second week of lactation (when both dams and offspring were treated) were analyzed for concentration of the test material. Due to low concentrations in the 0.4 mg/mL formulation during the second week of lactation, two additional analyses were done on the following week. For these analyses, samples were taken directly into volumetric flasks in order to avoid losses during transfer. Four samples were taken at each analysis, with two samples analyzed from each formulation. If the initial analysis required confirmation, then the remaining two samples were analyzed; otherwise, the samples were frozen as a contingency.

Results:

Homogeneity analysis: The mean concentration of top, middle, and bottom samples of the 0.4 mg/mL formulation (stored 0, 2 or 6 hours) for the first trial was 69-160% of the nominal concentration with a coefficient of variation (C.V.) of 1.91-68.7%. During trials two and three, the concentrations were 93-99% of the nominal value with a C.V. of 0.34-3.51%. The mean concentration of three trials of the 10 mg/mL formulation was 77-95% of the nominal value with a C.V. of 1.27-9.92%.

Stability analysis: The concentrations of the 0.4 mg/mL formulation stored for one or three days were 96% (C.V. 1.21%) and 85% (C.V. 1.78%) of the nominal value, respectively. The concentrations of the 10 mg/mL formulation stored for one or three days were 96% (C.V. 1.67%) and 95% (C.V. 1.30%) of the nominal value, respectively.

Concentration analysis: The percentages of the nominal concentration for the first two analyses during the treatment period were 82-87%, 85-93% and 92% for the 0.4, 2 and 10 mg/mL formulations, respectively. During the third analyses, the concentration of the 0.4 mg/mL formulation was 65% of the nominal value. On repeat analysis, it was 87% of the nominal 0.4 mg/mL concentration.

The analytical data indicated that the homogeneity and stability of Fluazinam were adequate. Doses to the animals were acceptable for the mid- and high-dose groups; however, the low-dose group may have been under dosed during some weeks.

C. OBSERVATIONS:

1. *In-life observations:*

a. Maternal animals:

1) **Clinical observations**: Females were observed cage-side for mortality, moribundity and clinical signs of toxicity at least twice daily during the study. A physical examination was performed on GDs 0, 6, 13 and 20, on LDs 1, 7 and 21 and then weekly to study termination. Detailed daily observations were recorded immediately before dosing, as each animal was returned to its home cage, at the end of dosing each group, between 1-2 hours after completion of dosing of all groups, and as late as possible in the working day. Individual maternal body weight was recorded on GDs 0, 3, 6, 10, 14, 17 and 20, daily until parturition and then weekly to study termination (Days 28, 35, 42, 49, 56 and 63). On Day 35, some body weight recordings were omitted in error. Food consumption (g/rat/day) was measured for the periods GDs 0-5, 6-9, 10-13, 14-16 and 17-19 and LDs 1-3, 4-6, 7-10, 11-13, 14-16 and 17-20.

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

i) **Functional observational battery (FOB):** On GDs 12 and 18 and LDs 6 and 18, twelve females per group were subjected to FOB testing. On post partum days (PPDs) 35, 45 and 60, ten females per group were also examined. Observations were made prior to dosing on treatment days. Observations made in the hand and in the arena (653 x 500 mm divided into six sections of equal size) were as follows:

	Functional observations[B] 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.

In addition, other sensory and reflex responses were assessed which included the following parameters: approach response, touch response, startle reflex, tail pinch, righting reflex, pupil closure reflex, landing foot splay, temperature and grip strength. Landing foot splay was not performed during gestation. Grip strength was measured using Mecmesin Portable Force Indicators; three trials each for the forelimbs and hindlimbs were conducted.

ii) **Motor activity testing:** Motor activity was evaluated in the same animals used for the FOB assessments on GD 15 and PPD 60. Activity was monitored for 60 minutes (10 six-minute intervals). Each animal was placed into a plastic cage and an automatic system incorporating a series of infra-red light sources and detectors was used to monitor activity. Low beam detectors set 5 cm above the cage floor on GD 15 and 6 cm on PPD 60 were considered to monitor ambulatory activity while high level beam detectors monitored rearing activity. Habituation was evaluated as a decrement in activity over consecutive intervals of each session.

iii) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on LD 19 and PPD 58 using the same animals tested for FOB assessments. The animals were tested in an automated system (Columbus Responder-X Response Monitor). Individual animals were placed into a soundproof container and allowed an acclimation period of 15 minutes. A series of 50 startle stimuli consisting of white noise at approximately 118 decibels (dB) of 40 millisecond (ms) duration was presented. The inter-stimulus interval was 12 seconds. Background noise throughout acclimation and testing was white noise at approximately 70 dB.

iv) **Learning and memory testing:** Learning and memory assessments were performed using a Morris maze on LD 16 and PPD 61 and consisted of a series of three trials on each of four consecutive days. The maze consisted of a circular pool constructed of white plastic (140 cm diameter and 45 cm deep) filled with water at a temperature of approximately 29^0C with an opacifier added. A platform 10 cm square was located at a fixed point in the pool, concealed approximately 1.5 cm below the water surface. Three starting points were identified at the perimeter of the pool and a number of visual cues outside the pool were available to assist with learning. On each testing day, each animal was tested in three consecutive trials. On the first trial, the rat was placed on the escape platform for 30 seconds prior to testing. The animal was then placed in the water at the perimeter of the pool and allowed a maximum of 90 seconds to swim to the platform. A different starting point was used for each trial. The time (latency) to reach the platform was recorded along with the number of quadrants (sectors) of the pool crossed. The rat was allowed to remain on the platform for 30 seconds after each trial. If the animal failed to find the platform within 90 seconds, it was placed on the platform for 30 seconds and a latency of 90 seconds was recorded.

b. Offspring:

1) **Litter observations:** All litters were examined at approximately 24 hours after birth (Day 1 of age) and then daily thereafter until weaning. The day of completion of parturition was

designated as PND 0. Mortality and changes in litter size were recorded from PND 1 to weaning and on PND 28. On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). The sex ratio of each litter was recorded on PNDs 1, 4 (before and after culling) and 21. Detailed daily observations were recorded immediately before dosing, as each animal was returned to its home cage, at the end of dosing each group, between 1-2 hours after completion of dosing of all groups, and as late as possible in the working day.

Surviving pups were weighed on PND 1, 4 (before culling) and 7-21. They were also weighed when vaginal patency and preputial separation were first evident.

2) **Developmental landmarks:** Beginning on postnatal day 32, male offspring were examined daily for preputial separation. Beginning on postnatal day 28, female offspring were examined daily for vaginal patency. The age of onset and the offspring body weight at attainment were recorded.

3) **Postweaning observations:** A detailed physical examination was performed on selected offspring on PND 28 and then weekly until termination (days 35, 42, 49, 56 and 63). Body weight was measured on PND 28 and then weekly until study termination.

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

i) **Functional observational battery (FOB):** On PNDs 4, 11, 21, 35, 45, and 60, a minimum often offspring/sex/group were examined outside the home cage in a FOB assessment. The behavioral assessments were customized for the offspring=s developmental stage. Where possible, the same offspring from each litter were examined. On PND 4, each selected offspring was placed in a clear Perspex arena (approximately 300 x 200 mm with side walls of approximately 25 mm) and offspring were tested for one minute. The following parameters were assessed: surface righting reflex, maximum pivoting angle, maximum distance traveled and number of sections entered. Righting reflex was performed using the same methodology as for parental females. Maximum pivoting angle was estimated relative to the original starting position in multiples of 45E comparing body alignment against marked lines on the arena floor. The next 45E angle was only scored if the body alignment reached or exceeded the angle. Animals exceeding a full 360E turn were scored as 360E. The maximum distance traveled (cm) from the animal=s starting point was assessed using marked concentric circles on the arena floor, when the animal=s head or body reached or crossed over the marked line. A section entry was achieved when both feet entered or crossed a line into a new section marked on the arena floor.

On PND 11, the following parameters were assessed: surface righting reflex, grooming, activity count (number of sections entered, as described above), rearing count and urination. On PNDs 21, 35, 45 and 60, observations in the arena, in the hand and manipulations were performed as described for parental females.

ii) **Motor activity testing:** Motor activity was evaluated in up to 12 pups/sex/dose, using one male or one female per litter, on PNDs 13, 17, 21 and 60. Activity was monitored for 60 minutes (ten six-minute intervals), as described for parental females, except low beam detectors to monitor ambulatory activity were set 2.5 cm above the cage floor.

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iii) **Sensory function (quantitative)**: Evaluation of sensory function was assessed by auditory startle habituation and pre-pulse inhibition of startle on PNDs 23/24 and 58. One set of up to 12 offspring/sex/dose, using one male or one female per litter, were assessed for habituation. Another set of 12/sex/dose was assessed for pre-pulse inhibition. Where possible, the same offspring from each litter were used for both time points but no offspring was tested for both parameters. Auditory startle habituation was tested using the same methodology described for parental females. For pre-pulse inhibition of startle testing, animals were tested using the same automated system. Animals were allowed a 15 minute acclimation period after being placed in a soundproof chamber. Mean auditory startle amplitude and latency to peak response were recorded during a randomized sequence of 40 trials comprising the following:

Startle stimulus - 50 ms pulse of white noise of approximately 118 dB (10 trials)
Pre-pulse - 50 ms of white noise at 85 dB and startle stimulus (10 trials)
Pre-pulse - 50 ms of white noise at 85 dB only (10 trials)
No stimulus (10 trials)

The pre-pulse preceded the stimulus by 100 ms and the inter-stimulus interval was 12 seconds. Background noise throughout testing, including acclimation, was white noise at approximately 70dB.

iv) **Learning and memory testing**: Assessment of learning and memory, consisting of a series of three trials conducted on each of four consecutive days, was performed on PNDs 23/24 and 61. Different sets of up to 12 offspring/sex/group, using one male or one female per litter, were assessed at each testing. Occasional offspring may have been subjected to other behavioral testing but no offspring were assessed for learning and memory at both time points. The same methodology was used as described for parental females, except the pool and platform were smaller for the animals tested on PNDs 23/24.

2. **Postmortem observations**:

a. **Maternal animals**: For each group, twelve parental females were sacrificed on LD 21, with ten being selected for neuropathology necropsy (perfusion) procedures. The remaining twelve females in each group were killed on PPD 66, with ten being selected for perfusion procedures. Adults selected for perfusion procedures received an intraperitoneal injection of a lethal dose of barbiturate followed by perfusion of the fixative via the aorta. Adults not selected for perfusion procedures were killed by carbon dioxide asphyxiation.

After the lethal injection of barbiturate and confirmation of death, the heart was exposed, the circulatory system was flushed and the fixative (glutaraldehyde and paraformaldehyde) was gravity-fed from a reservoir. The animals were then subjected to a macroscopic examination, which included assessment of the number of implantation sites in the uterus. For one control and one intermediate dose female, errors were made in the recording of implantation sites. The brain was transected from the spinal cord above the first cervical spinal nerve and weight, length and width measurements were taken. The length of the brain was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum. Brain width was measured at the widest part of the cerebral hemispheres. Selected tissues, any abnormalities and the carcass were retained in fixative (glutaraldehyde and paraformaldehyde). For peripheral nerves, only right-sided specimens were removed; left-

sided specimens were retained *in situ*. Slides of tissues from ten females in the control and high dose group at the Day 21 and Day 66 necropsy were prepared and stained with hematoxylin and eosin. The brain and spinal cord were stained with Luxol fast blue. The sciatic and tibial nerves were stored at 4^0C prior to embedding in resin, then sectioned and stained with toluidine blue. Tissues sectioned and examined by light microscopy included the following:

Tissue	Area sectioned
Brain	**Coronal sections:** Olfactory lobes Forebrain Cerebrum, hippocampus, thalamus, hypothalamus Cerebrum, tectum, tegmentum **Mid-sagittal sections:** Cerebellum, pons
Spinal cord	Transverse and longitudinal sections at cervical and lumbar swellings
Dorsal root ganglia	One cervical and one lumbar level
Dorsal root fibers	Two longitudinal sections, at one cervical and one lumbar levels
Ventral root fibers	Two longitudinal sections, at one cervical and one lumbar levels
Eyes (retina)	One longitudinal section each
Optic nerves	One longitudinal section each
Skeletal muscle (gastrocnemius)	One transverse section
Sciatic nerve (right)	Longitudinal and transverse sections at the sciatic notch and the mid-thigh
Tibial nerves (right)	Longitudinal and transverse sections at the knee. Longitudinal and transverse sections of calf muscle branch(es)

Morphometric measurements were conducted on LD 21 and PPD 66 to measure the thickness of the brain at four selected areas:

Neocortex (measurement 1): The distance from the pial surface to the top of the white matter was measured along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits its greatest thickness.

Hippocampus (measurement 2): The greatest dorsal-ventral thickness of the hippocampus was measured.

Corpus callosum (measurement 3): The thickness of the corpus callosum at the midline was measured.

Cerebellum (measurement 4): The width of the pyramis folia was measured perpendicular to its long axis at the midpoint between its tip and base.

b. ***Offspring***: Tissues from selected offspring that were perfused and sacrificed on PNDs 21 and 66 were examined using the same methodology described for parental females, except only the brain and spinal cord were processed and examined for PND 21 animals.

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D. DATA ANALYSIS:

1. **Statistical analyses:** For body weight and body weight gain, food consumption, litter size, survival indices, sex ratio, FOB data, motor activity, startle data (habituation and pre-pulse), Morris maze data, brain weight, brain measurements at necropsy, brain morphometry, the following sequence of statistical tests was used. If 75% of the data (across groups) were the same value, then a frequency analysis was applied. Treatment groups were compared using a pairwise Fisher's Exact Test for each group against the control. If the Bartlett test for variance homogeneity was not significant at the 1% level, then a parametric analysis was applied. If the F1 test for monotonicity of dose-response was not significant at the 1% level, Williams test for a monotonic trend was applied. If the F1 test was significant, Dunnett's test was performed instead. If the Bartlett test was significant at the 1% level, then logarithmic and square-root transformations were used. If the Bartlett test was still significant, non-parametric tests were applied. If the H1 test for monotonicity of dose response was not significant at the 1% level, Shirley's test for a monotonic trend was applied.

   At 10 mg/kg/day, litter size and mortality after Day 7 were affected by the number of mortalities due to suspected dosing errors. A trend test was not considered appropriate. A Bartlett test indicated that non-parametric analyses should be applied. The data were initially analyzed using a Kruskal-Wallis test. A significant difference between the groups was apparent at the 5% level; therefore, treatment groups were compared using Steel's test.

   For brain weight data and startle amplitudes, analysis of covariance was initially performed using terminal body weight as covariate. If the within group relationship between organ weight/startle amplitude and body weight was significant at the 10% level, then the above treatment comparisons were made on adjusted group means in order to allow for the influence of body weight on the parameter.

   For Morris maze data, the reciprocal of the mean of three trials was analyzed, for the sector count the square root of the mean of three trials was analyzed and for the number of fails frequency analysis or non-parametric analysis was used.

   For categorical data, such as pathological findings, the proportion of animals was analyzed using Fisher's Exact test for each treated group versus the control.

2. **Indices:**

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

   $$\text{Gestation index} = \frac{\text{number of live litters born}}{\text{number pregnant}} \times 100$$

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

Post-implantation survival index = $\frac{\text{total number offspring born}}{\text{total number of uterine implantation sites}} \times 100$

Live birth index (%) = $\frac{\text{number of live pups on Day 1}}{\text{total number of pups born}} \times 100$

Viability index (%) = $\frac{\text{number of live pups on day 4 preculling}}{\text{number of live pups on Day 1}} \times 100$

Lactation index (%) = $\frac{\text{number of live pups on day 7/21}}{\text{number of live pups on day 4 post-culling}} \times 100$

3. Positive and historical control data: Positive control and method validation studies were previously submitted and the following studies are currently either under review or a review is planned: MRIDs 46484602 (auditory startle response), 46484603 (FOB), 46484601 (Morris water maze), 45308301 (motor activity), 45308302 (Morris water maze and auditory startle response), 43680415 and 43680414 (both for FOB) and 44447801 and 44447802 (both for neuropathology).

4. Historical control data were submitted with the current study for PND 4 and 11 arena observations, including surface righting reflex, maximum pivoting angle, maximum distance traveled, activity and rearing count. Morris maze performance data were included for PNDs 23/24 and 61∀3. Historical control data on brain weight, length and width for PND 66 were also submitted.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** All parental females survived to the scheduled sacrifices. There were no treatment-related clinical signs of toxicity, except for a single female at 10 mg/kg/day that salivated after dosing on one occasion.

2. **Body weight and food consumption**: Selected group mean body weight, body weight gain and food consumption values for pregnant and nursing dams are summarized in Table 2. Mean body weight during gestation and lactation was not affected by treatment. Mean body weight gain for GDs 6-14 was significantly decreased (86% of control value) in females at 10 and 50 mg/kg/day and during GDs 6-20 at 50 mg/kg/day (90% of control value). Mean body weight gain during lactation was not affected by treatment. Food consumption (g/rat/day) in treated females during gestation was comparable to controls but was significantly decreased at 10 (86-90% of control value) and 50 mg/kg/day (89-93% of control value) during lactation. Mean body weight and body weight gain post weaning (days 28-63) were similar between the treated and control groups.

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

Observations/study interval	Dose (mg/kg/day)			
	0	2	10	50
Gestation (n= 24)				
Body wt. Gestation day 0 (g)	281 ±20	284 ±24	287 ± 23	284 ± 22
Body wt. Gestation day 6 (g)	308 ± 20	312 ± 24	315 ±23	312 ± 23
Body wt. Gestation day 14 (g)	344 ± 21	351 ± 24	346 ± 25	343 ± 24
Body wt. Gestation day 20 (g)	426 ±27	436 ±28	428 ±28	418 ± 32
Wt. gain gestation days 0-6	27 ± 5	28 ± 8	28 ±7	28 ±7
Wt. gain gestation days 6-14	36 ±7	39 ±4	31* ±9 (14)	31* ± 8 (14)
Wt. gain gestation days 6-20 (g)	118 ±15	124 ±12	113 ±13	106** ± 15 (10)
Food consumption gestation days 0-5 (g/rat/day)	28 ± 2	28 ±3	28 ±2	28 ± 2
Food consumption gestation days 6-9 (g/rat/day)	28 ±3	28 ± 3	27±3	27 ±2
Food consumption gestation days 17-19 (g/rat/day)	30 ± 3	30 ±3	30 ± 3	29 ± 3
Lactation (n=23-24)				
Body wt. lactation day 1 (g)	325 ± 20	333 ± 24	327 ± 22	324 ± 23
Body wt. lactation day 4 (g)	333 ±22	342 ± 22	336 ± 19	333 ± 24
Body wt. lactation day 7 (g)	337 ± 23	349 ± 25	341 ±20	340 ±22
Body wt. lactation day 14 (g)	354 ± 20	364 ± 26	349 ±19	352 ± 19
Body wt. lactation day 21 (g)	348 ±23	361 ± 27	347 ± 17	349 ± 23
Wt gain lactation days 1-21(g)	23 ±15	28 ± 12	20 ± 17	25 ± 7
Food consumption lactation days 1-3 (g/rat/day)	41 ± 7	40 ± 7	37 ±4	37* ± 6 (10)
Food consumption lactation days 7-10 (g/rat/day)	60 ±5	59 ± 6	54* ± 9 (10)	54** ± 9 (10)
Food consumption lactation days 17-20 (g/rat/day)[b]	81 ± 10	79 ± 8	70** ± 16 (14)	72** ± 10 (11)
Post Weaning (n=10-12)				
Body wt. post partum day 28 (g)	311 ± 19	322 ± 27	311 ± 22	302 ± 18
Body wt. post partum day 63 (g)	325 ± 24	337 ± 33	326 ±25	318 ± 19
Wt. gain post partum days 28-63 (g)	14 ± 13	14 ± 10	14 ±9	16 ± 10

[a]Data obtained from pages 103-110, MRID 46534401.
[b] Includes food consumed by offspring.
Number in parentheses is percent of control, calculated by reviewer.

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3. **Reproductive performance**: Results for the maternal animals are summarized in Table 3. The gestation index was 100% for the control and all treated groups. The mean duration of gestation in treated groups was comparable to the control group.

TABLE 3. Reproductive performance[a]

Observation	Dose (mg/kg/day)			
	0	2	10	50
Number mated	24	24	24	24
Number pregnant	24	24	24	24
Gestation index (%)	100	100	100	100
Intercurrent deaths	0	0	0	0
Mean (±SE) gestation duration (days)[b]	22.3	22.3	22.3	22.4

[a] Data obtained from page 144, MRID 46534401.
[b] Calculated by the reviewer from data on page 144 of MRID 46534401.

4. **Behavioral assessment**:

a. **Functional observational battery**: FOB testing (in the hand, in the arena and manipulations) was conducted on GDs 12 and 18, LDs 6 and 18 and PPDs 35, 45 and 60. On LD 18 and PPDs 35, 45 and 60, activity counts in the arena were lower than controls at 50 mg/kg/day but did not attain statistical significance. Landing foot splay was significantly lower on day 35 post partum at 10 and 50 mg/kg/day but was comparable to the control group prior to and after that day. For example, landing foot splay was 135±16, 135±10, 117±20 and 124±12 for the control, low, mid and high dose groups. Neither of these alterations is considered toxicologically significant.

b. **Motor/locomotor activity**: Total motor (high and low beam) activity data for GD 15 and PPD 60 are presented in Table 4. Subsession data for motor activity are included in Table 5. At 10 and 50 mg/kg/day, high beam scores were significantly lower during the first six minutes on PPD 60 but there was no dose response. On this day, high beam scores were also significantly lower during the last six minutes. Motor activity habituation was evident on both testing days.

TABLE 4. Motor activity data- group mean (±S.D.) scores (beam breaks) in F_0 rats [a]

Day	Dose (mg/kg/day)			
	0	2	10	50
Motor Activity - High Beam Level				
GD 15	199.8 ± 59.7	239.8 ± 67.0	257.6 ±152.2	234.3 ±99.0
PPD 60	238.3 ± 95.3	180.3 ± 92.6	178.8 ±145.1	166.3 ± 91.8
Motor Activity - Low Beam Level				
GD 15	771.2 ± 179.0	939.3 ± 231.7	962.2 ± 344.0	825.8 ± 235.4
PPD 60	565.2 ± 208.0	519.8 ± 170.5	565.7 ± 228.2	563.5 ± 254.2

[a] Data obtained from pages 138-139, MRID 46534401.
GD = Gestation Day; PPD = Post Partum Day

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N=10

TABLE 5: Sub-session motor activity (# beam breaks/6 minute interval) in F_0 rats [a]

Interval	Dose (mg/kg/day)			
	0	2	10	50
	GD 15 - High Beam			
6	103.4 ± 24.7	99.9 ± 16.6	102.7 ± 45.2	100.3 ± 27.5
12	38.6 ± 17.8	44.0 ±12.5	49.9 ±35.1	55.8 ±20.6
18	19.5 ± 14.7	23.8 ±13.2	24.6 ± 18.5	23.5 ± 21.2
24	9.4 ± 14.9	16.9 ± 15.9	21.6 ± 13.5	15.1 ± 17.3
30	8.3 ±7.6	18.2 ± 16.8	19.2 ±20.7	15.4 ±17.7
36	12.8 ±20.8	13.2 ± 22.2	12.2 ±12.9	8.7 ± 12.8
42	4.8 ± 16.1	8.6 ±13.3	15.4 ± 31.6	3.3 ± 10.3
48	0.0 ± 0.0	10.4* ± 18.9	3.2 ± 7.4	4.8 ±5.2
54	0.0 ± 0.0	1.9 ±3.3	2.5 ±4.5	4.9 ±11.6
60	3.0 ±10.4	2.9 ±10.1	6.4 ±11.6	2.5 ± 8.1
	GD 15 - Low Beam			
6	271.8 ± 39.3	262.6 ± 58.2	257.7 ± 58.9	242.6 ± 46.9
12	157.0 ± 28.1	168.4 ± 49.0	143.9 ± 36.0	173.9 ± 39.2
18	95.0 ± 35.4	126.5 ±29.7	111.2 ±38.7	119.6 ± 42.7
24	67.2 ± 60.4	91.7 ±62.4	111.7 ±51.1	76.7 ± 57.1
30	64.5 ± 47.9	86.2 ± 58.6	108.4 ±68.6	62.2 ± 48.0
36	60.7 ± 65.3	75.1 ± 70.7	67.6 ±50.8	45.0 ±61.6
42	33.8 ±70.1	47.8 ±50.8	77.1 ± 65.9	36.3 ±48.7
48	3.3 ±4.9	36.7 ± 39.7	34.0 ± 48.2	20.1 ± 38.9
54	1.8 ±3.7	23.9 ± 33.3	18.1 ±29.0	23.0 ± 42.3
60	16.3 ±35.2	20.5 ± 39.0	32.6 ± 44.0	26.4 ±39.5
	PPD 60 - High Beam			
6	99.2 ±29.1	88.4 ±29.6	69.8* ± 24.3	75.3* ± 26.8
12	48.1 ± 23.7	43.7 ± 42.0	29.4 ±23.7	25.8 ±23.7
18	23.6 ±19.0	14.1 ±19.3	23.9 ±36.0	15.4 ±18.9
24	14.2 ±21.0	6.8 ±13.8	17.8 ±22.3	10.0 ± 13.1
30	13.1 ±5.6	12.8 ±20.8	9.4 ±24.9	8.5 ±10.7
36	2.8 ±6.2	4.9 ±10.9	6.7 ± 18.5	11.6 ±15.4
42	0.3 ±0.7	3.4 ± 6.8	2.4 ±4.6	7.0 ± 15.8
48	13.1 ±30.3	2.4 ±6.3	2.9 ±5.0	3.4 ±7.9
54	14.6 ±33.3	1.5 ± 3.2	7.9 ±11.1	9.3 ±29.1

Interval	TABLE 5: Sub-session motor activity (# beam breaks/6 minute interval) in F_0 rats [a]			
	Dose (mg/kg/day)			
	0	2	10	50
60	9.3 ±11.9	2.3 ± 5.5	8.6 ±19.1	0.0** ±0.0
	PPD 60 - Low Beam			
6	193.3 ± 46.1	210.1 ±43.2	185.7 ± 40.7	202.7 ±58.1
12	113.7 ±46.9	98.6 ±46.3	100.9 ±44.5	96.4 ±48.0
18	64.6 ± 46.4	58.3 ± 51.7	64.3 ± 47.9	52.4 ±58.2
24	40.8 ± 32.1	25.4 ± 25.6	56.8 ± 49.0	44.2 ± 43.4
30	39.1 ± 33.8	42.5 ±43.5	23.6 ±36.4	38.2 ± 29.2
36	24.4 ±28.7	31.0 ± 37.8	19.5 ± 25.2	50.8 ±44.6
42	9.6 ±18.9	19.4 ± 18.3	18.1 ±29.3	33.0 ± 43.0
48	26.1 ±46.4	11.2 ±18.7	29.2 ± 39.1	14.9 ±19.6
54	24.4 ±39.7	11.5 ± 20.4	24.8 ±37.2	22.1 ± 58.1
60	29.2 ± 30.7	11.8 ±15.8	42.8 ± 51.8	8.8 ±12.4

[a] Data were obtained from pages 138-139, MRID 46534401.
GD = Gestation Day; PPD = Post Partum Day
N = 12 for GD 15 and 10 for PPD 60
* Significantly different from control group, $p<0.05$
** Significantly different from control group, $p<0.01$

c. **Auditory startle reflex habituation**: The interval values for amplitude and latency measurements on LD 19 and PPD 58 are included in Table 6. No treatment-related differences from the control group were observed.

TABLE 6. Mean (±S.D.) interval auditory startle peak amplitude (g) and latency to peak (msec) in F_0 rats[a]

Trial Number	Dose (mg/kg/day)			
	0	2	10	50
LD19				
Latency to peak (ms)				
38361	13.9 ± 2.4	14.0 ± 1.5	15.4 ± 3.1	14.2 ± 2.5
38675	13.5 ±1.7	12.9 ±1.3	15.2 ±2.4	14.3 ±2.1
21-30	13.3 ±1.5	13.1 ±1.6	14.2 ± 2.3	13.8 ± 1.7
31-40	12.8 ± 1.4	13. ±2.4	13.1 ±1.0	13.3 ±1.4
41-50	12.6 ± 1.0	12.8 ± 1.5	12.9 ±1.1	13.1 ± 1.3
Peak amplitude (g)				
38361	798.7 ± 215.3	787.0 ±184.8	748.2 ±179.0	773.3 ± 180.2
38675	696.5 ±205.6	680.9 ±138.6	697.8 ± 165.2	702.4 ±100.1
21-30	660.0 ± 148.9	649.8 ±161.9	599.9 ± 70.3	662.4 ± 148.2
31-40	654.5 ± 140.0	649.7 ±162.3	595.0 ±100.3	674.4 ± 166.6
41-50	655.9 ± 164.0	619.3 ±138.6	625.6 ±165.4	684.7 ±151.7
PPD 58				
Latency to peak (ms)				
38361	15.6 ± 4.4	15.7 ± 1.9	16.3 ±3.8	14.7 ± 2.7
38675	13.7 ±1.9	14.1 ± 2.6	13.9 ±1.1	14.6 ±1.8
21-30	13.9 ± 2.4	13.8 ± 2.0	13.9 ± 3.0	13.2 ± 1.1
31-40	13.9 ± 2.8	14.4 ±2.7	14.3 ±3.6	14.9 ± 3.1
41-50	15.6 ± 4.2	13.9 ±3.5	14.9 ±3.4	14.4 ± 3.7
Peak amplitude (g)				
38361	841.3 ±237.0	747.4 ±150.7	817.6 ±209.9	795.7 ±261.0
38675	678.2 ±184.1	701.1±158.3	699.3 ±163.3	798.0 ±277.7
21-30	641.3 ±151.6	639.9 ± 164.1	629.1 ±149.1	703.8 ± 168.6
31-40	665.2 ± 140.2	620.6 ± 129.4	600.6 ± 142.2	658.2 ± 146.6
41-50	651.4 ± 166.7	650.0 ±141.8	587.8 ±142.0	627.7 ± 147.6

[a]Data obtained from pages 140-141, MRID 46534401.
GD = Gestation Day; PPD = Post Partum Day
N = 12 for LD 19 and 10 for PPD 58

d. **Learning and memory testing**: Watermaze performance data are presented in Tables 7 (LD 16) and 8 (PPD 61). No treatment-related effects were observed. During testing beginning on PPD 61, females at 50 mg/kg/day had a significantly higher number of failed trials on Day 2 but there was no dose response.

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TABLE 7. Watermaze performance data (mean ±S.D.) in F_0 rats on LD 16[a]

Parameter	Dose (mg/kg/day)			
	0	2	10	50
Day 1				
Trial time (secs - mean of 3 trials)	80.2 ± 11.2	78.8 ±12.5	80.1 ± 13.8	74.9 ± 13.2
Number of failed trials (90 secs)	2.3 ± 0.7	2.3 ± 0.7	2.3 ± 0.8	2.1 ± 0.7
Percentage with at least 1 failed trial (90 secs)	100.0	100.0	100.0	100.0
Sector entries (mean of 3 trials)	15.4 ± 2.7	15.2 ± 2.6	14.9 ± 3.0	15.9 ± 4.0
Day 2				
Trial time (secs - mean of 3 trials)	55.1 ± 22.4	48.0 ± 19.7	53.3 ± 23.2	47.5 ± 20.5
Number of failed trials (90 secs)	1.1 ±0.9	0.7 ±0.9	1.3 ± 1.1	0.6 ± 0.7
Percentage with at least 1 failed trial (90 secs)	70.0	50.0	80.0	50.0
Sector entries (mean of 3 trials)	12.1 ± 3.6	11.7 ± 3.7	11.8 ±3.9	10.7 ±3.4
Day 3				
Trial time (secs - mean of 3 trials)	36.6 ±24.6	30.6 ±21.6	37.7 ± 22.8	36.5 ± 19.9
Number of failed trials (90 secs)	0.5 ±0.8	0.2 ± 0.6	0.5 ± 0.8	0.5 ± 0.7
Percentage with at least 1 failed trial (90 secs)	30.0	10.0	30.0	40.0
Sector entries (mean of 3 trials)	8.7 ± 4.2	8.0 ± 4.2	9.0 ±4.8	8.6 ± 3.9
Day 4				
Trial time (secs - mean of 3 trials)	40.5 ±24.2	28.4 ±30.4	38.0 ±22.7	17.7* ±11.1
Number of failed trials (90 secs)	0.7 ±0.9	0.6 ±1.1	0.5 ±1.0	0.1 ± 0.3
Percentage with at least 1 failed trial (90 secs)	50.0	30.0	30.0	10.0
Sector entries (mean of 3 trials)	9.5 ±5.6	7.8 ±6.8	10.1 ±4.4	5.1 ± 3.0

[a]Data obtained from 142, MRID 46534401.
LD = Lactation Day
* Significantly different from control group, $p<0.05$

TABLE 8. Watermaze performance data (mean ±S.D.) in F_0 rats on PPD 61[a]

Parameter	Dose (mg/kg/day)			
	0	2	10	50
Day 1				
Trial time (secs - mean of 3 trials)	66.1 ± 11.4	62.3 ± 7.3	68.2 ±16.7	66.3 ± 16.2
Number of failed trials (90 secs)	1.4 ±0.8	1.3 ±0.5	1.8 ± 0.8	1.7 ± 0.7
Percentage with at least 1 failed trial (90 secs)	90.0	100.0	100.0	100.0
Sector entries (mean of 3 trials)	14.6 ±2.8	13.2 ±2.7	15.2 ±2.9	14.1 ± 4.1
Day 2				
Trial time (secs - mean of 3 trials)	31.7 ±18.5	39.9 ±13.1	36.6 ± 20.2	46.4 30.9
Number of failed trials (90 secs)	0.2 0.4	0.6 0.5	0.3 0.7	1.1* 1.2
Percentage with at least 1 failed trial (90 secs)	20.0	60.0	20.0	60.0
Sector entries (mean of 3 trials)	8.8 4.4	10.3 3.1	10.3 4.3	9.4 3.8
Day 3				
Trial time (secs - mean of 3 trials)	19.6 ± 12.4	21.6 ±15.6	32.9 ±19.9	34.8 ± 21.9
Number of failed trials (90 secs)	0.1 ±0.3	0.2 ± 0.4	0.4 ± 0.5	0.5 ± 0.7
Percentage with at least 1 failed trial (90 secs)	10.0	20.0	40.0	40.0
Sector entries (mean of 3 trials)	6.3 ±3.4	5.9 ±3.0	8.8 ± 4.4	8.3 ±4.0
Day 4				
Trial time (secs - mean of 3 trials)	23.2 ± 12.8	19.7 ± 14.9	25.2 ± 20.2	25.5 ± 17.0
Number of failed trials (90 secs)	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.1 ± 0.3
Percentage with at least 1 failed trial (90 secs)	0.0	0.0	30.0	10.0
Sector entries (mean of 3 trials)	6.8 ± 3.4	5.8 ±2.7	6.8 ±4.5	6.9 ± 3.3

[a]Data obtained from page 143, MRID 46534401.
PPD = Post Partum Day
* Significantly different from control group, $p<0.05$

In adults assessed on postpartum day 61, the trial times showed an apparent increase on day 2 and day 3. The values were 31.7±18.5, 39.9±13.1, 36.6±20.2 and 46.4±30.9 for day 2 and 19.6±12.4, 21.6±15.6, 32.9±19.9 and 34.8±21.9 secs for the control, low, mid and high dose groups, respectively. However, the data are variable as indicated by the large standard deviations and not robust enough to determine there is an effect of treatment.

5. Postmortem results:

a. **Brain weight and measurements:** Mean brain weight and measurement data are presented in Table 9. No treatment-related differences between treated and control groups in brain weight and brain measurements (length and width) were observed.

TABLE 9. Mean (±S.D.) Brain Weight and Measurement Data in F_0 rats[a]				
Parameter	Dose (mg/kg/day)			
	0	2	10	50
LD 21				
Brain weight (g)	1.91 ± 0.08	2.00 ±0.12	1.95 ±0.16	2.01 ±0.08
Brain length (mm)	21.0 ± 0.5	20.9 ± 0.6	20.9 ±0.7	21.1 ± 0.6
Brain width (mm)	14.8 ±0.4	15.0 ±0.5	15.2 ±0.8	15.4 ± 0.5
PPD 66				
Brain weight (g)	2.05 ±0.09	2.05 ± 0.08	2.05 ±0.11	2.06 ± 0.08
Brain length (mm)	21.2 ±0.4	21.2 ±0.9	21.0 ±0.6	21.1 ± 0.4
Brain width (mm)	15.2 ±0.5	15.1 ± 0.5	15.2 ±0.4	15.1 ± 0.5

[a] Data obtained from pages 201-202 and 204-205, MRID 46534401.
N=10
LD = Lactation Day; PPD = Post Partum Day

b. **Macroscopic examination**: No gross lesions were observed at necropsy on LD 21 or PPD 66.

c. **Neuropathology**:

1) ***Microscopic examination***: Microscopic examination was limited to control and high dose animals on LD 21 and PPD 66. Minimal or slight degenerative changes in peripheral nerves were observed in both control and treated groups. No lesions were reported in the brain indicative of vacuolation of the grey matter. .

2) **Brain Morphometry:** No treatment-related changes were observed in four morphometric measurements taken on LD 21 and PPD 66. Data are presented in Table 10.

TABLE 10: Mean (±S.D.) morphometric measurements (mm) in F_0 rats[a]		
Parameter	Dose (mg/kg/day)	
	0	50
LD 21		
Neocortex	1.47 ± 0.15	1.51 ± 0.13
Hippocampus	1.92 ±0.12	1.82 ±0.20
Corpus Callosum	0.34 ±0.07	0.34 ±0.04
Cerebellum	0.80 ± 0.07	0.81 ± 0.07
PPD 66		
Neocortex	1.66 ± 0.07	1.66 ± 0.10
Hippocampus	1.92 ± 0.12	2.02 ± 0.19
Corpus Callosum	0.31 ± 0.03	0.32 ± 0.04
Cerebellum	0.84 ±0.04	0.87 ± 0.08

[a] Data were obtained from pages 203 and 206, MRID 46534401.
N=10
LD = Lactation Day; PPD = Post Partum Day

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 11. At 50 mg/kg/day, the number of mean implantations and litter size (total and live) on PND 1 and PND 4 (pre-cull) was non-significantly decreased (92-95% of the control value). Offspring survival after culling in this group was comparable to the control group. At 10 mg/kg/day, a single incidence of total litter loss occurred on PND 3 and as a result, live litter size was significantly decreased during PNDs 7-11. Many of the deaths in this group were the result of a technical dosing error. The lactation index for the 10 mg/kg/day group was lower on PNDs 7 and 21, with statistical significance achieved on PND 21. The sex ratio in treated groups was comparable to the control group on all days on which it was calculated.

   The abdomen of offspring from one litter in the 50 mg/kg/day group appeared large on PNDs 3-5 and dark on PNDs 3-11; these pups also showed a distended abdomen beginning on PND 12 but this resolved by PND 19. Another four offspring from three different 50 mg/kg/day litters showed dark and/or distended abdomen between PNDs 9-15. According to the study report, similar signs were observed at 50, 100 and 200 mg/kg/day in the preliminary study. In the 10 mg/kg/day group, five offspring from three litters also had a dark abdomen with one pup also showing distention. However, four of these animals were found to have a ruptured esophagus at necropsy.

   There were a total of 7, 7, 31 and 13 pups which died during the post cull phase of the lactation period (refer to page 380-383) for the control, low, mid and high dose groups respectively. The macropathology report (page 208), however, reports that only 4, 2, 24 and 10 pups dying in each study group were examined. Of the animals examined, there were 1, 0, 8 and 0 animals for which the report states there was evidence of ruptured esophagus to indicate a dosing error. The report does not define the cause of death for the remainder of the pups and this is 16 pups in the mid dose group. Thus, the higher rate of pup death in the mid dose group can only be partially accounted for by dosing error and there is no explanation as to why the mid dose group has a higher rate of dosing errors. However, since there is no similar increase in the next higher dose, the increased rat of pup death in the mid dose group
   is noted but not attributed to the test material.

TABLE 11. Litter size and viability of F_1 rats [a]

Observation	Dose (mg/kg/day)			
	0	2	10	50
No. of litters	24	24	24	24
Total number of pups born[b]	368	388	361	345
Total no. of pups alive[b]	360	384	355	337
Total no. of pups found dead[b]	8	4	6	8
Implantations (mean S.D.)	16.4 ± 1.6	16.7 ±2.4	16.0 ± 2.0	15.6 ± 2.5 (5
Post implantation survival index	93.1	94.7	93.3	91.2
Total litter size (mean S.D.)	15.3 ± 1.8	16.2 ± 2.1	15.0 ± 1.6	14.4 ± 2.3 (6)
Sex ratio (% of males) - Day 1	49.5 ± 8.4	52.2 ±11.9	51.3 ±15.0	48.3 ± 9.5
Live litter size (mean S.D.)				
Day 1	15.0 ±1.6	16.0 ± 2.1	14.8 ± 1.5	14.0 ± 2.5 (7)
Day 4 (precull)	14.9 ± 1.5	15.5 ± 2.5	14.5 ± 1.4	13.7 ± 2.8 (8)
Day 4 (postcull)	8.0 ±0.0	8.0 ± 0.0	8.0 ± 0.0	7.9 ± 0.4
Day 7	7.9 ± 0.3	7.9 ± 0.3	7.7 ± 0.9	7.7 ± 0.8
Day 11	7.7 ±0.8	7.8 ±0.5	6.8* ±1.5	7.5 ± 1.3
Day 14	7.7 ± 0.8	7.7 ±0.6	6.7* ±.5	7.5 ±1.3
Day 18	7.7 ±0.8	7.7 ±0.6	6.7* ±1.5	7.4 ±1.3
Day 21	7.7 ±0.8	7.7 ±0.6	6.7* ±1.5	7.4 ±1.3
Live Birth Index	98.1	99	98.9	97.4
Viability Index	99.5	97	97.7	97
Lactation Index				
Day 7	99.0	98.4	96.7	97.8
Day 21	96.4	96.4	83.2*	93.4

[a] Data obtained from pages 145-147, MRID 46534401.
[b] Calculated by the reviewer from data on pages 380-383
* *Statistically significantly different from control group, $p<0.05$*
N = 23-24
Number in parentheses is percent of control, calculated by reviewer.

2. **Body weight:** Selected mean preweaning pup body weight and body weight gain data are presented in Table 12. Mean body weight was significantly decreased in males and females at 10 (89-94% of control value) and 50 mg/kg/day (84-94% of control value) prior to weaning. Mean body weight gain was significantly decreased in males and females at 10 (76-96% of control value) during the first week of lactation and at 50 mg/kg/day (65-84% of control value) during the entire lactation period. During the post-weaning period (PNDs 28-63), mean body weight was significantly decreased in males and females at 10 (93-97% of control value) and 50 mg/kg/day (85-93% of control value) (Table 13). Body weight gain in treated groups was comparable to the control group.

TABLE 12. Selected mean (±S.D.) pre-weaning pup body weight and body weight gain [a]

PND	Dose (mg/kg/day)							
	0	2	10	50	0	2	10	50
	Males				Females			
	Body Weight (g)							
1	6.8 ±0.5	6.6 ±0.5	6.5 ±0.5	6.5 ±0.6	6.5 ±0.5	6.3 ±0.6	6.1** ±0.5 (6)	6.1** ±0.5 (6)
4[b]	9.2 ± 0.9	8.7 ± 1.3	8.3* ± 1.1 (10)	8.2** ±1.0 (11)	8.9 ±0.8	8.4 ± 1.3	7.9** ± 1.1 (11)	7.9** ± 1.1 (11)
4[c]	9.4 ± 0.7	8.9 ±1.3	8.5** ± 1.2 (10)	8.2** ± 1.0 (13)	9.0 ± 0.7	8.5 ± 1.3	8.0** ± 1.1 (11)	7.9** ± 1.1 (12)
11	25.2 ±2.3	24. ±4 2.9	23.8 ± 3.9 (6)	21.9** ±3.5 (13)	24.4 ± 1.9	23.5 ± 2.7	22.3* ±3.8 (9)	21.1** ± 3.8 (14)
18	42.1 ±2.9	40.9 ± 3.6	40.7 ± 6.4	36.5** ±4.6 (13)	41.0 ± 2.3	39.8 ±3.1	38.4* ±5.9 (6)	34.9** ±5.2 (15)
21	50.2 ±4.1	48.7 ± 5.7	47.4 ± 8.2 (94)	42.5** ± 5.6 (15)	48.7 ± 3.3	47.4 ±5.2	45.0 ± 8.1 (8)	40.9** ±6.2 (14)
	Body Weight Gain (g)							
1-4	2.6 ±0.4	2.3 ±0.9 (12)	2.0* ± 0.8 (23)	1.7**± 0.7 (35)	2.5 ± 0.5	2.2 ± 0.8	1.9* ± 0.9 (24)	1.9** ±0.8 (24)
1-7	8.6 ± ±1.4	8.2 ± 1.8	7.6 ± 1.9 (12)	7.2** ± 2.0 (16)	8.3 ± 1.3	7.8 ±1.6	7.2* ± 1.9 (13)	7.0* ± 1.9 (16)
1-21	43.4 ± 3.8	42.2 ± 5.3	40.9 ±7.9 (6	36.0** ± 5.2 (17)	42.2 ± 3.1	41.1 ±4.8	38.9 ± 7.8 (8)	34.8** ±5.9 (18)

[a] Data obtained from pages 148-151, MRID 46534401.
[b] Before standardization (culling).
[c] After standardization (culling).
PND = post-natal day
N=23-24
* Statistically significantly different from control, p# 0.05
** Statistically significantly different from control, p#0.01
Number in parentheses is percent of control, calculated by reviewer.

TABLE 13. Selected mean (±S.D.) post-weaning pup body weight and body weight gain [a]

PND	Dose (mg/kg/day)			
	0	2	10	50
Males				
		Body Weight (g)		
28	87 ± 6	85 ± 9	81* ±12 (7)	74** ± 9 (15)
35	135 ± 8	141 ±14	129 ±18	124* ±14 (8)
56	329 ±21	329 ±26	317 ± 33	304** ±31 8)
63	375 ± 23	378 ± 28	362* ±35 (3)	352** ±32 (6)
		Body Weight Gain (g)[b]		
28-35	48	56	48	50
35-56	194	188	188	180 (7)
28-63	288	293	281	278
Females				
Body Weight (g)				
28	80 ±5	78 ±10	75** ±11 (6)	69** ±8 (14)
35	122 ± 8	117 ± 15	116 ± 15	110** ±11 (10)
56	213 ± 14	210 ± 17	206* ±19 (3)	197** ± 17 (8)
63	233 ± 17	229 ±19	226 ± 19	217** ±18 (7)
Body Weight Gain (g)[b]				
28-35	42	39	41	41
35-56	91	93	90	87
28-63	153	151	151	148

[a] *Data obtained from pages 152-153 and 156-157, MRID 46533401.*
[b] Calculated by the reviewer without standard deviations.
PND = post-natal day
* 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**: These data are presented in Table 14. The mean age of completion of balano-preputial separation was significantly delayed in males at 10 and 50 mg/kg/day. Mean body weight at completion was comparable to the control group. No treatment-related effect on the completion of vaginal opening in females was observed. Body weight in females at 50 mg/kg/day was significantly decreased (92% of control value) at the time of completion of vaginal opening.

TABLE 14. Mean (±S.D.) age (days) at completion of sexual maturation [a]				
Parameter	Dose (mg/kg/day)			
	0	2	10	50
N (M/F)	59/60	60/60	55/53	56/57
Preputial separation				
mean age	45.3 ± 1.9	45.6 ± 2.2	46.5** ± 2.4	48.4** ±2.8
%change			+2.6%	+6.8%
day change			+1.2 days	+3.1 days
mean body weight	234.5 ±21.3	239.0 ± 25.0	234.4 ± 21.0	239.0 ± 28.6
Vaginal opening				
mean age	36.0 ±2.4	35.8 ±3.1	36.3 ±3.1	37.1 ± 3.8
mean body weight	129.0 ±16.3	123.4 ±14.1	123.0 ± 17.0	119.1** ±8.7 (8)

[a] Data obtained from page 160, MRID 46534401.
** Statistically significantly different from control, p<0.01.
Number in parentheses is % of control value, calculated by reviewer.

b. **Developmental landmarks**: Other endpoints were not monitored.

4. **Behavioral assessment**:

a. **Functional observational battery**: Selected FOB data are presented in Table 15. On PND 4, female offspring at 10 and 50 mg/kg/day (both with mean scores of 2.5) had higher mean scores than the control (1.9) or low dose (2.2) groups meaning there were more animals with grades 2 or 3 but all groups had animals with grade 3 severity (see table). On that day, a tendency for male and female offspring in all treated groups to have lower mean maximum pivoting angles, distance traveled and activity than the control group was observed but there was no statistical significance or dose response in males. On PND 11, mean activity counts for females at 10 and 50 mg/kg/day were slightly lower than the control group but there was no statistical significance or dose response in males.

On PND 21, mean activity counts were significantly lower in females at 10 (score 4.5) and 50 (score 4.8) mg/kg/day (vs. 6.8 in the control). Rearing counts were significantly decreased in males at 50 mg/kg/day (3.9 vs. 7.9 in the control) and females at 10 (3.5) and 50 (3.7) mg/kg/day vs. 8.1 in the control on PND 21. Also on PND 21, the number of males and females with one or both pupils failing to dilate (total of 4 for each sex vs. only one in the other groups) during the pupil reflex testing was increased. Forelimb (-11%), $p < 0.01$) and hindlimb (-18%, $p < 0.01$) grip strength were significantly lower in females at 50 mg/kg/day on PNDs 21 and 35, respectively.

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TABLE 15. Number of F_1 rats with selected FOB signs [a]

Observation	Dose (mg/kg/day)							
	Males				Females			
	0	2	10	50	0	2	10	50
PND 4								
Surface righting reflex								
Grade 1	4	3	7	4	5	3	0	1
Grade 2	2	3	1	2	2	4	5	4
Grade 3	6	6	4	6	4	5	6	7
Mean	2.2	2.3	1.8	2	1.9	2.2	2.5	2.5
Max. pivoting angle (E)								
0	3	6	8	6	3	6	3	6
45	3	2	3	2	3	3	5	2
90	1	3	0	1	1	1	2	4
135-180	3	0	0	2	2	1	1	0
225-360	2	1	1	1	2	1	0	0
Mean	120.0	52.5	41.3	60.0	94.7	63.8	49.1	37.5
Max. distance traveled (cm)								
0	9	10	11	8	5	8	8	9
2	2	1	0	1	3	3	3	3
4	0	1	1	3	2	1	0	0
6	1	0	0	0	1	0	0	0
Mean	0.8	0.5	0.3	1.2	1.8	0.8	0.5	0.5
Activity (number of sections)								
0	3	4	6	4	2	4	2	3
1-5	6	7	5	6	6	6	8	8
6-10	1	1	0	2	3	2	1	1
11-15	1	0	1	0	0	0	0	0
16-20	1	0	0	0	0	0	0	0
Mean	4.1	2.0	1.9	2.4	3.4	2.3	2.3	1.8
PND 11								
Activity count								
0	1	1	0	3	0	2	1	0
1-2	2	3	2	4	2	2	3	4
3-5	5	4	6	2	5	3	4	5
6-10	3	1	2	2	1	2	2	2
11-15	1	2	1	0	3	2	0	1
16-20	0	1	1	1	1	1	1	0
Mean	4.9	5.8	6.3	4.2	6.8	6.0	4.5	4.8
PND 21								
Activity count								
Mean	14.5	12.8	8.0**	4.8**	13.4	11.6	8.3	8.8

TABLE 15. Number of F_1 rats with selected FOB signs [a]

Observation	Dose (mg/kg/day)							
	Males				Females			
	0	2	10	50	0	2	10	50
Rearing count Mean	7.9	10.8	5.7	3.9*	8.1	5.7	3.5**	3.7**
Forelimb grip strength (kg) Mean (S.D.)	0.18 ±0.01	0.18 ± 0.02	0.17± 0.03	0.17 ± 0.04	0.18 ± 0.01	0.18 ± 0.02	0.18 ± 0.03	0.16** ±0.01
Both pupils failed to dilate	0	0	1	2	0	0	0	1
Left pupil failed to dilate	0	1	0	2	1	1	0	3
Right pupil failed to dilate	0	0	1	0	0	0	0	0
PND 35								
Hindlimb grip strength (kg) Mean (S.D.)	0.21 ± 0.04	0.24 ± 0.03	0.21 ± 0.03	0.19 ± 0.03	0.22 ± 0.03	0.21 ±0.03	0.20 ± 0.04	0.18* ± 0.03

[a] Data obtained from pages 164-176, MRID 46534401.
* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

b. **Motor/locomotor activity**: Total motor activity data for PNDs 13, 17, 21 and 60 are presented in Table 16. Subsession data for motor activity are included in Tables 17 (males) and 18 (females). No treatment-related effects on total motor activity (high and low beam) was observed. Low beam activity for females at day 17 was lower (see table). Otherwise sporadic statistically significant differences from the control group were observed in all treated groups, but there was no consistency or dose response. Habituation was observed on PNDs 22 and 60 but not on PNDs 13 and 17.

TABLE 16. Total motor activity data- group mean (±S.D.) scores (beam breaks) in F_1 rats [a]

PND	Dose (mg/kg/day)			
	0	2	10	50
Males				
Motor Activity - High Beam Level				
13	14.3± 21.5	17.7± 19.6	13.8± 32.3	14.0± 18.4
17	45.4±59.8	80.4± 72.0	29.6± 39.9	33.0± 46.9
22	102.7± 80.5	117.5± 71.8	104.5±117.1	87.9± 60.5
60	364.5± 67.1	389.4± 126.8	414.8±156.6	309.4± 99.4
Motor Activity - Low Beam Level				
13	434.4±168.9	714.0± 456.0	471.6± 182.4	567.7± 347.6
17	698.0± 459.0	875.2±487.0	448.9± 368.6	641.5± 428.9
22	436.2± 270.7	440.9±234.6	370.9± 399.6	507.3± 293.3
60	1220.5± 296.5	1212.4±331.6	1159.8± 308.4	1024.7± 382.5
Females				
Motor Activity - High Beam Level				
13	25.8±43.8	31.4±41.3	9.9±16.7	24.5±36.6
17	123.1± 110.1	105.8±115.5	105.7± 132.5	66.4±94.0
22	97.8± 79.1	116.6± 88.0	102.2±60.1	96.6± 66.7
60	391.6±123.0	432.4±71.2	400.4± 112.9	406.4± 133.2
Motor Activity - Low Beam Level				
13	544.1±427.1	582.3± 489.6	461.8± 322.5	612.5± 258.9
17	940.5 ±564.3	962.6± 871.4	747.8± 590.7	719.5±639.9
22	379.7 ±234.7	375.3±227.9	496.8±354.9	430.9± 242.3
60	1135.9 ±85.5	1189.3 ±317.3	1146.6± 141.2	1119.5±342.3

[a] *Data obtained from pages 177-184, MRID 46534401.*
PND = post-natal day
N=11-12

TABLE 17. Sub-session motor activity (# beam breaks/6 minute interval) in male F_1 rats[a]				
Interval	Dose (mg/kg/day)			
	0	2	10	50
PND 13 - High Beam				
6	1.4± 3.3	2.2±2.4	1.7± 3.7	0.1±0.3
12	0.8± 0.3	2.8±6.0	1.8 ±3.4	2.5±7.7
18	0.3± 0.8	1.8±2.9	0.5± 1.4	2.5± 6.9
24	0.4±1.2	1.1± 2.5	1.9 ±6.3	0.4±1.4
30	0.8 ±1.9	2.3 ±2.7	1.4 ±4.3	1.1± 2.5
36	0.8±2.9	1.0 ±2.2	0.3± 0.6	2.3±3.8
42	0.6± 1.5	0.2± 0.4	3.0± 9.2	3.5± 7.3
48	5.3±11.5	1.2 ±2.3	1.3±3.2	0.8± 1.7
54	1.8±4.0	2.7± 5.0	0.0± 0.0	0.6 ±1.7
60	2.0±6.9	2.6± 6.3	1.9± 4.3	0.3± 0.6
PND 13 - Low Beam				
6	61.8± 44.9	86.7±33.7	53.8 ±26.6	47.6± 44.7
12	40.0±38.3	66.6±41.7	50.6± 34.1	46.8± 59.6
18	52.6± 47.9	90.5±62.1	59.8± 38.0	34.9±33.2
24	31.8± 27.8	71.7±65.1	45.8 ±40.3	50.4± 39.0
30	22.0± 21.2	90.2* ±61.4	43.3*± 32.3	81.3**± 64.5
36	44.1 ±33.6	57.3 ±3.7	46.8± 52.8	72.5± 73.1
42	53.1± 52.0	50.3±48.6	58.4 59.0	75.6 73.6
48	43.946.4	73.4 82.8	42.0± 39.5	43.8 ±46.8
54	51.4±42.9	61.8±65.1	32.0± 29.5	70.8 ±75.6
60	33.7±39.0	65.6±73.5	39.0± 43.4	43.9± 61.0
PND 17 - High Beam				
6	2.5 ±3.5	2.2 ±3.2	2.3± 5.1	2.3±3.5
12	4.3± 6.6	5.5±4.4	4.5± 5.3	1.1± 1.5
18	4.4±5.7	8.4±9.0	2.3 ±2.6	2.6 ±4.5
24	4.8 ±7.3	14.7 ±12.8	3.2 ±4.7	1.1± 1.7
30	2.3 ±5.8	10.2* ±10.0	2.0±3.5	3.9 ±7.1
36	7.1 ±15.1	8.3± 10.2	2.4±4.3	4.1± 9.0
42	5.8± 9.4	9.0 ±13.4	3.8± 8.6	4.5± 8.7
48	7.8±10.8	9.6±12.3	5.6±9.5	6.3±9.1
54	3.7±5.9	4.0 ±8.1	3.3 ±11.5	3.8 ±7.5
60	2.7± 5.7	8.7 ±12.8	0.1 ±0.3	3.3 ±5.8
PND 17 - Low Beam				
6	72.8 ±32.6	68.0 ±3.8	67.7± 39.5	58.2 ±30.1

TABLE 17. Sub-session motor activity (# beam breaks/6 minute interval) in male F_1 rats[a]				
Interval	Dose (mg/kg/day)			
	0	2	10	50
12	91.4± 55.4	101.8±53.8	64.5± 47.3	56.4 ±58.0
18	81.0± 63.6	115.2±53.9	61.6± 49.1	79.1± 51.0
24	91.8 ±73.5	126.2± 58.7	39.2± 46.7	62.8± 42.3
30	72.3± 68.6	115.9± 79.1	52.7± 60.0	52.0± 57.1
36	70.1±62.5	71.6±63.0	40.0±55.5	52.8 ±49.5
42	67.8 ±1.5	78.7 ±7.2	44.1± 53.1	73.2 ±84.5
48	58.4±73.2	81.0 ±100.2	42.4 ±55.4	78.1± 80.3
54	49.5± 52.8	47.4± 65.6	27.1 ±45.0	62.6± 85.4
60	42.9± 54.0	69.4± 83.0	9.8 ±22.8	66.4± 81.5
PND 22 - High Beam				
6	22.4± 19.1	37.5 ±16.1	26.3±21.1	14.1± 17.2
12	16.3 ±17.9	22.3 ±19.4	18.4 ±18.2	16.5 ±13.9
18	5.6±9.0	19.2±16.8	11.9 ±13.5	12.5± 13.1
24	6.6± 9.1	7.2 ±10.1	4.8±10.0	5.3± 5.4
30	9.6 ±14.5	7.1±10.0	5.8 ±16.8	8.3 ±9.0
36	7.7 ±13.2	6.1± 8.4	6.5 ±10.4	7.1± 7.7
42	9.6 ±4.7	8.3 ±13.4	7.3± 13.6	4.0± 4.4
48	7.5± 13.6	5.8± 8.7	8.9± 15.8	3.6± 4.4
54	9.4±14.7	3.1± 6.9	9.9± 21.1	11.9± 18.0
60	8.1± 12.4	1.1± 3.4	4.6± 9.1	4.6 ±7.5
PND 22 - Low Beam				
6	113.8± 54.1	137.8±42.4	103.9± 63.3	89.2± 69.8
12	55.6 ±51.5	65.9 ±33.1	47.1±34.6	88.7± 59.4
18	36.0± 47.1	55.5 ±43.3	52.5± 61.1	61.7± 49.7
24	31.8 ±35.8	31.6± 39.4	26.5± 40.9	40.7± 38.2
30	38.3±45.4	50.7±52.3	21.8± 43.4	48.8± 51.6
36	28.5±42.0	30.9± 42.8	24.3 ±37.1	45.9± 31.3
42	35.7± 45.5	26.3± 32.6	22.6± 42.4	38.4 ±31.0
48	31.3± 50.0	19.9± 30.0	23.2± 42.6	21.9± 26.1
54	31.3± 42.8	14.8±22.7	27.4± 52.9	34.6±43.3
60	34.1±38.9	7.7± 14.5	21.7±39.5	37.3± 35.1
PND 60 - High Beam				
6	145.5± 35.8	115.8± 39.4	128.3 ±25.7	102.1** ±35.3
12	64.0 ±26.4	64.8 ±20.5	77.4± 25.1	70.6± 28.8
18	44.8 ±26.5	61.5±36.1	48.0± 29.6	29.5 ±20.5

TABLE 17. Sub-session motor activity (# beam breaks/6 minute interval) in male F_1 rats[a]				
Interval	Dose (mg/kg/day)			
	0	2	10	50
24	26.9 ±20.9	30.3±17.2	49.0± 38.3	12.5±14.7
30	34.3±23.6	31.5±23.5	26.8± 23.4	17.2± 31.2
36	13.7± 12.3	23.8± 28.7	28.3± 33.5	25.8 ±33.8
42	10.0± 11.7	17.8± 20.0	15.8 ±17.6	12.7± 22.4
48	7.4 ±10.8	24.6±23.7	16.8 ±28.5	16.9± 23.9
54	9.4± 18.7	8.7± 16.6	5.3 ±15.5	11.5 ±22.0
60	8.5±20.0	10.6 ±17.0	19.0±40.2	10.5 ±16.9
PND 60 - Low Beam				
6	313.3± 58.5	301.1± 63.4	303.6± 51.3	287.7± 40.0
12	213.9± 53.7	190.5± 48.8	186.7± 35.2	168.2* ±53.2
18	157.6±65.7	150.0± 48.0	152.8 ±65.7	118.8 ±73.7
24	115.0± 64.6	129. ±8 47.3	138.6 ±83.5	66.7±37.4
30	124.5±44.2	107.7 ±71.9	91.4 ±54.9	65.6* ±64.0
36	85.3 ±2.5	87.0± 81.1	95. ±5.6	73.5± 61.0
42	74.5±69.5	81.6±48.7	73.7± 45.7	62.6 ±76.0
48	60.4 ±61.9	85.7± 66.5	52.8± 61.8	67.7± 64.7
54	43.5 ±46.0	40.3± 34.9	18.3 ±24.2	45.1± 76.5
60	32.6±62.1	38.8 ±53.7	46.8 ±50.1	68.6 ±72.5

[a] Data were obtained from pages 177, 179, 181 and 183, MRID 46534401.
N = 11-12
* Significantly different from control group, p<0.05
** Significantly different from control group, p<0.01

TABLE 18. Sub-session motor activity (# beam breaks/6 minute interval) in female F_1 rats[a]				
Interval	Dose (mg/kg/day)			
	0	2	10	50
PND 13 - High Beam				
6	3.0 ±4.2	2.1± 4.0	0.5± 0.8	2.6±5.0
12	2.0± 4.4	3.6 ±5.8	0.6 ±1.8	3.7±10.6
18	1.6± 4.0	5.3± 9.4	0.5± 1.5	3.7± 6.6
24	1.5± 3.1	3.8±5.8	2.8± 7.8	2.4± 4.9
30	1.8± 5.7	3.1± 7.1	0.5±1.2	1.2 ±2.2
36	1.8± 4.9	1.8 ±4.1	1.5± 3.7	1.8± 3.3
42	1.5 4.3	1.8 3.9	0.1 0.3	3.8 7.1
48	2.2± 3.6	0.8 ±2.9	1.4± 3.6	1.5v2.8
54	6.2 ±10.9	1.3± 2.3	0.2± 0.6	1.3 ±2.7

TABLE 18. Sub-session motor activity (# beam breaks/6 minute interval) in female F_1 rats*				
Interval	Dose (mg/kg/day)			
	0	2	10	50
60	4.3± 8.1	7.9 ±14.3	2.0± 5.4	2.7± 6.6
PND 13 - Low Beam				
6	49.9± 28.4	67.3 ±45.2	62.3± 40.4	91.7* ±43.7
12	46.6±43.2	53.3±47.5	63.3± 48.6	62.3± 33.6
18	47.5± 51.9	61.6 ±37.6	51.6± 45.1	76.1± 53.0
24	47.0±41.7	62.8±48.4	65.5±43.6	51.3 ±31.7
30	57.4± 66.7	79.8 ±79.3	57.4 ±49.9	59.8±46.2
36	37.4±50.7	53.8±64.7	44.2 ±40.0	59.3± 41.1
42	35.3± 41.5	49.1± 83.7	23.5 ±56.0	63.7± 42.7
48	52.9 ±59.6	43.3± 74.2	19.6± 26.4	70.4± 67.4
54	83.9±89.5	33.4 ±26.6	32.9± 48.8	44.5± 58.4
60	86.2±103.3	78.1± 108.3	41.5± 52.9	33.6 ±43.7
PND 17 - High Beam				
6	5.4 ±5.9	5.2 ±9.6	2.5 ±3.6	1.8v2.1
12	8.7±6.4	10.2±13.9	6.9 ±10.2	1.9**± 2.2
18	14.5±12.0	10.9±14.3	5.8 ±11.6	4.9± 5.6
24	12.3± 12.9	12.5 ±14.9	11.5± 19.2	6.2±8.4
30	15.0± 17.5	8.3 ±12.3	11.5±17.9	4.9± 8.7
36	12.1± 17.8	11.4± 11.5	11.4± 15.9	4.3± 6.6
42	16.4± 17.2	9.2± 10.7	13.5 ± 21.0	9.9± 16.5
48	14.3± 12.9	9.8± 13.4	23.5± 34.5	10.9± 18.3
54	17.2±20.3	11.3± 20.6	6.4± 13.6	11.8± 19.8
60	7.3± 9.3	17.1± 21.3	12.7± 18.4	9.8± 18.8
PND 17 - Low Beam				
6	86.4 ±53.6	84.2 ±49.2	62.5 ±42.6	54.1± 39.5
12	108.2±78.8	92.5±57.2	79.4±71.2	71.9 ±50.0
18	112.2± 58.0	101.3± 67.8	75.5± 70.2	88.2± 61.7
24	98.9±55.9	110.6±108.8	78.4± 73.7	72.2± 81.3
30	94.5 ±72.5	101.9 ±101.4	66.9 ±76.7	68.1± 62.7
36	85.8 ±74.0	109.3± 124.5	79.3± 68.2	69.8± 81.8
42	105.5± 87.0	94.2 ±122.3	79.8± 87.4	68.0± 83.0
48	94.0±76.5	87.1 ±105.1	93.6±81.1	81.7± 98.8
54	98.3± 88.5	79.6 ±119.0	56.3± 73.9	79.3 ±106.1
60	56.7±71.2	102.1±119.6	76.1 ±96.9	66.3± 89.3
PND 22 - High Beam				

TABLE 18. Sub-session motor activity (# beam breaks/6 minute interval) in female F_1 rats*				
Interval	Dose (mg/kg/day)			
	0	2	10	50
6	35.8±21.5	29.3 ±23.1	25.7±17.2	23.9± 18.4
12	16.9 ±14.3	25.3±33.1	21.6 ±19.7	16.3± 13.1
18	4.3± 6.4	13.1±12.5	14.4 ±16.1	15.2± 21.8
24	6. ±2.8	8.5 ±13.7	2.0±5.1	6.5±9.2
30	3.5 ±7.8	3.3±5.6	4.6± 8.5	8.0± 9.8
36	8.9±12.8	6.5±12.4	5.5± 10.6	7.6±9.1
42	5.5± 12.1	5.4±11.1	2.7 ±5.4	9.8 ±13.1
48	4.3±9.5	10.1± 24.0	5.8 ±13.4	3.9 ±9.3
54	6.7 ±11.4	8.7±13.3	13.6±20.3	4.1±9.6
60	5.3±11.1	6.5 ±15.4	6.2±9.0	1.3 ±4.6
PND 22 - Low Beam				
6	132.1±60.7	105.4± 70.8	113.8± 50.1	115.0± 68.9
12	70.0± 32.1	68.3± 48.2	76.6±50.2	70.4± 45.7
18	26.3±23.8	48.3 ±37.4	66.2±57.0	48.3± 50.4
24	18.5± 35.4	30.7±51.4	32.4 ±41.8	31.7±33.7
30	14.7± 21.4	15.6 ±25.5	41.0±69.1	38.8± 43.9
36	41.0 46.1	19.5 40.4	30.3 55.0	38.7 40.6
42	21.0 ±34.4	18.8 ±30.1	26.5± 45.5	37.0± 40.4
48	12.8± 24.9	25.0± 45.8	32.7± 55.2	18.2 ±32.6
54	23. ±5.1	21.0 ±39.0	41.8± 56.4	20.8 ±37.0
60	20.1±39.0	22.9 ±44.6	35.5± 35.2	12.0 ±19.8
PND 60 - High Beam				
6	123.3± 24.8	123.1± 27.6	112.0± 33.8	113.8± 25.5
12	64.9± 19.9	76.6±26.1	72.1± 16.9	66.9±25.4
18	32.0 ±24.7	54.1±31.4	42.8±32.2	54.8± 37.5
24	25.8±23.8	34.0±27.1	27.3± 23.7	34.3± 26.6
30	30.3 ±6.3	38.5± 26.3	40.7± 30.1	22.8±26.5
36	36.0 ±26.3	42.0±33.2	26.4± 31.3	25.8± 25.3
42	24.3±19.5	27.4± 22.5	23.8±19.8	20.2±29.0
48	17.3± 20.6	15.6± 16.3	14.2±19.8	26.8± 25.3
54	19.7±30.6	10.3± 13.1	17.4 ±19.2	16.1± 20.3
60	17.9±21.9	10.8 ±21.3	23.7± 23.9	25.0± 28.4
PND 60 - Low Beam				
6	290.2±49.3	310.8 ±55.5	272.2± 54.8	294.0± 44.8
12	168.3±50.5	183.5±63.5	183.2±39.9	178.5± 47.1

TABLE 18. Sub-session motor activity (# beam breaks/6 minute interval) in female F_1 rats[a]				
Interval	Dose (mg/kg/day)			
	0	2	10	50
18	117.5± 70.4	137.4± 60.3	122.5± 54.7	141.7±51.8
24	81.5± 47.1	107.4 ±58.3	112.1 ±56.7	87.1 ±45.8
30	89.3± 53.5	88.3± 34.2	95.5± 56.1	68.9± 53.2
36	112.3± 50.1	99.5±60.0	91.9±53.9	76.8± 64.1
42	76.8 ±43.8	80.3±67.9	74.0±50.6	53.8± 46.4
48	75.1±60.3	90.0±83.2	47.7± 50.1	72.0± 75.8
54	55.0± 68.4	56.1± 63.6	59.2 ±46.0	72.9 ±8.2
60	69.8±61.4	35.9±42.8	88.4±64.0	73.8± 59.2

[a] Data were obtained from pages 178, 180, 182 and 184, MRID 46534401.
N = 11-12
* Significantly different from control group, $p<0.05$
** Significantly different from control group, $p<0.01$

c. **Auditory startle reflex habituation**: The amplitude and latency data for PNDs 23/24 and 58 are presented in Tables 19 (males) and 20 (females). Analysis of the auditory startle peak amplitude using data unadjusted for body weight indicated that the males in the high dose on PND 23/24 were significantly affected (refer to supplemental study report in MRID No.: 47018301, page 6). The peak amplitude both with and without pre-pulse stimulus were lower in males at day 23/24. See also Appendix to this DER.

Also, in females at 50 mg/kg/day, a significant increase in latency was observed on PND 23/24 during the 41-50 minute interval. On PND 58, a significant decrease in latency during the 1-10minute interval was reported in females at 50 mg/kg/day. On PND 58, peak amplitude was significantly decreased in all treated female groups during the last three intervals.

Pre-pulse inhibition of auditory startle data are presented in Tables 21 (males) and 22 (females).

TABLE 19. Mean (±S.D.) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 male rats[a]

Trial Number	Dose (mg/kg/day)			
	0	2	10	50
PND 23/24 - Latency to peak (ms)				
1-10	18.9± 6.4	20.6± 5.7	20.2± 6.8	21.9± 6.0
11-21	15.2± 3.7	14.0 ±1.4	15.5 ±2.5	13.9± 1.7
21-30	14.8± 2.4	14.8 ±2.7	13.9± 1.6	15.1± 5.4
31-40	13.8 ±2.2	14.3± 2.1	14.3± 2.1	14.3± 1.5
41-50	14.3± 2.0	14.0± 1.8	15.2 ±2.9	13.9± 1.9
PND 23/24 - Peak amplitude (g)				
1-10	145.6± 31.9	138.4± 26.4	127.5 ±28.1	116.3± 19.4
11-21	150.2±43.6	143.0± 35.7	129.3± 25.7	113.6± 13.4 *(a)
21-30	151.2± 43.2	140.2 ±30.7	128.3±26.1	120.8 ±20.94 *(a)
31-40	142.9 ±31.0	139.9±39.3	135.1±33.3	112.6 ±18.74 *(a)
41-50	143.6±33.90	133.6±34.8	131.0± 32.9	114.9 ±21.44 *(a)
PND 58 - Latency to peak (ms)				
1-10	14.0 ±2.8	19.1 ±7.1	14.1 ±2.6	17.3± 4.9
11-21	13.6 ±3.4	12.3 ±1.2	12.8± 1.7	13.6± 2.4
21-30	13.1± 3.5	14.2± 3.0	13.2±4.8	15.8 ±5.4
31-40	13.3±3.5	13.8± 2.3	15.4 ±5.7	16.8 ±5.7
41-50	13.6 ±3.2	15.3± 3.7	12.4±2.0	15.0±3.1
PND 58 - Peak amplitude (g)				
1-10	794.2 ±182.0	759.1± 140.9	745.4 ±235.5	735.4 ±202.4
11-21	723. ±53.0	666.3± 155.6	608.6± 162.5	644.0± 168.7
21-30	636.3± 142.8	621.0 ±139.7	527.6 ±142.4	577.2 ±115.7
31-40	635.5 ±129.1	615.9 ±116.7	553.6 ±119.2	564.8± 101.5
41-50	618.4 ±155.2	625.8 ±111.2	533.6± 119.2	573.9 ±32.1

[a]Data obtained from pages 185 and 187, MRID 46534401.
*(a) statistically significant unadjusted vale. Refer to MRID 47018301 page 6.

TABLE 20. Mean (±S.D.) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 female rats[a]

Trial Number	Dose (mg/kg/day)			
	0	2	10	50
PND 23/24 - Latency to peak (ms)				
1-10	17.4± 4.8	17.0±5.1	19.5 ±5.8	19.6± 8.5
11-21	14.0 ±.3	13.7 ±1.7	15.5 ±2.5	14.2± 2.5
21-30	14.0± 2.0	13.8 ±1.5	13.8±3.0	13.0± 1.0
31-40	13.5±1.3	13.7±1.2	14.9± 3.5	15.4 ±4.5
41-50	13.8 ±1.6	14.4± 3.0	15.8± 2.5	16.5*± 4.6
PND 23/24 - Peak amplitude (g)				
1-10	142.9± 29.2	126.1± 16.5	126.5± 24.6	128.2± 32.4
11-21	139.8 ±33.7	131.1 ±22.6	121.0± 24.4	119.5 ±38.8
21-30	140.5 ±30.7	127.3 ±23.8	125.8± 25.6	119.3 ±32.4
31-40	140.8 ±30.4	132.9 ±26.2	125.8± 22.7	120.0 ±28.7
41-50	135.8 ±4.4	127.6 ±25.4	121.2±19.9	119.4 ±32.7
PND 58 - Latency to peak (ms)				
1-10	21.0 ±9.1	16.6± 7.7	18.3 ±5.7	14.3*± 5.3
11-21	13.8 ±.0	12.9±2.8	14.8 ±4.6	13.2± 1.9
21-30	15.0 ±5.9	14.9±4.4	15.9 ±7.3	14.2 ±4.0
31-40	13.7± 4.4	14.7± 2.9	15.1± 3.2	15.9± 7.0
41-50	16.5±3.6	16.0 ±6.3	14.1± 2.5	15.3±5.0
PND 58 - Peak amplitude (g)				
1-10	596.9 ±230.5	447.0± 96.4	530.0 ±165.4	480.1± 143.9
11-21	560.2 ±218.3	404.5 ±77.1	469.5 ±106.5	443.8± 117.4
21-30	499.0 ±140.2	400.3* ±9.5	410.3* ±90.6	390.9* ±75.6
31-40	522.7 ±158.8	372.3**± 71.1	411.1**± 87.9	366.3** ±65.6
41-50	486.6 ±115.3	380.5* ±106.1	418.4* ±97.8	331.1** ±58.3

[a]Data obtained from pages 186 and 188, MRID 46534401.
* Significantly different from control group, $p<0.05$.
** Significantly different from control group, $p<0.01$.

TABLE 21. Mean (±S.D.) interval auditory startle pre-pulse inhibition peak amplitude (g) and latency to peak (msec) in F_1 male rats[a]

Trial Number	Dose (mg/kg/day)			
	0	2	10	50
PND 23/24 - Latency to peak (ms)				
Stimulus without pre-pulse	13.8 ±2.3	15.1 ±2.4	15.3 ±2.3	16.1± 4.3
Stimulus with pre-pulse	18.1± 7.5	16.4 ±3.9	17.5±3.5	17.5 ±3.9
PND 23/24 - Peak amplitude (g)				
Stimulus without pre-pulse	158.8± 37.5	148.5 ±42.4	139.4 ±30.5	120.3± 25.5*(a)
Stimulus with pre-pulse	134.8±33.9	126.7 ±36.7	119.9 ±26.0	101.5± 20.9 *(a)
Percentage inhibition	14.9 ±.2	14.0 ±10.1	13.8 ±5.8	14.3± 15.0
PND 58 - Latency to peak (ms)				
Stimulus without pre-pulse	12.6± 0.9	14.8 ±4.0	14.8± 3.1	15.3± 5.9
Stimulus with pre-pulse	13.5 ±1.9	19.2 ±5.8	16.1±4.8	16.6± 8.2
PND 58 -Peak Amplitude (g)				
Stimulus without pre-pulse	866.8±159.1	758.3 ±364.3	770.9± 213.0	676.5* ±124.9
Stimulus with pre-pulse	614.0 ±147.9	577.3±292.1	577.2 ±143.5	502.8± 94.1
Percentage inhibition	29.5±8.1	23.3 ±9.1	23.4 ±14.0	25.2± 9.1

[a]Data obtained from pages 189 and 191, MRID 46534401.
* Significantly different from control group, $p<0.05$
*(a) statistically significant $p < 0.05$ based on unadjusted values. Refer to MRID 47018301 page 8.

TABLE 22. Mean (±S.D.) interval auditory startle pre-pulse inhibition peak amplitude (g) and latency to peak (msec) in F_1 female rats[a]

Trial Number	Dose (mg/kg/day)			
	0	2	10	50
PND 23/24 - Latency to peak (ms)				
Stimulus without pre-pulse	15.4 ±2.9	16.2± 4.2	15.6± 6.2	14.6 ±1.8
Stimulus with pre-pulse	18.7 ±5.4	16.3± 3.2	18.0 ±6.4	16.0 ±3.0
PND 23/24 - Peak amplitude (g)				
Stimulus without pre-pulse	156.6± 41.1	133.2± 25.1	134.7±32.6	138.3± 25.6
Stimulus with pre-pulse	124.8± 33.6	108.6±20.1	117.9± 31.9	114.2 ±29.9
Percentage inhibition	19.0 ±16.1	18.0 ±8.1	12.4 ±12.2	17.6± 15.0
PND 58 - Latency to peak (ms)				
Stimulus without pre-pulse	16.4 ±4.9	16.3±5.7	15.0 ±3.2	16.9± 6.1
Stimulus with pre-pulse	20.8± 5.5	17.3±2.8	17.6 ±6.3	21.5± 6.5
PND 58 - Peak amplitude (g)				
Stimulus without pre-pulse	507.0±132.9	474.1± 141.4	506.7± 81.5	479.2± 138.7
Stimulus with pre-pulse	384.8± 97.7	334.8± 73.8	338.0± 69.0	339.3± 62.7
Percentage inhibition	23.1±12.3	27.4 ±11.2	32.8 ±10.6	26.7 ±11.2

[a]Data obtained from pages 190 and 192, MRID 46534401.

d. **Learning and memory testing:** The watermaze performance data are summarized in Tables 23 (males) and 24 (females). The control group demonstrated both learning and memory of the task. Males at 50 mg/kg/day had significantly increased swimming times and numbers of failed trials on Days 3 and 4 of the testing that began on PND 23/24. The time on Day 3 was outside the historical control range but the value on Day 4 was within the range. On PND 61, all treated males had significantly increased swimming times for Days 3 and 4. There was no dose response and all values were within the historical control range, except for the time on Day 4 for the 2 mg/kg/day group. Females at 50 mg/kg/day had a significantly decreased mean sector entries values on Day 2 of the Day 23/24 testing but is not considered an adverse effect.

TABLE 23. Watermaze performance data (mean ±SD) in male F_1 rats [a]

Parameter	Dose (mg/kg/day)			
	0	2	10	50
	PND 23/24			
Day 1				
Trial time (secs - mean of 3 trials)	60.9±16.2	65.9± 20.3	57.0± 16.2	73.6± 16.2
Number of failed trials (90 secs)	1.4± 0.8	1.6± 1.1	1.1± 0.9	2.0± 0.8
Percentage with at least 1 failed trial (90 secs)	91.7	83.3	72.7	100.0
Sector entries (mean of 3 trials)	18.5±4.6	16.8±5.0	15.6 ±3.1	16.6± 3.0
Day 2				
Trial time (secs - mean of 3 trials)	32.2± 18.0	53.2± 19.4	36.3 ±14.8	49.1± 24.4
Number of failed trials (90 secs)	0.3±0.5	0.7±0.8	0.4 ±0.5	0.8± 0.8
Percentage with at least 1 failed trial (90 secs)	25.0	50.0	36.4	63.6
Sector entries (mean of 3 trials)	11.2± 5.7	15.3±5.1	11.6± 4.9	13.2± 5.0
Day 3				
Trial time (secs - mean of 3 trials)	32.1± 21.4	29.7±19.2	35.9± 22.4	52.5*±23.9
Number of failed trials (90 secs)	0.3± 0.7	0.3± 0.7	0.3± 0.5	0.8 ±1.0
Percentage with at least 1 failed trial (90 secs)	25.0	25.0	27.3	54.5
Sector entries (mean of 3 trials)	11.1± 6.4	9.0±5.1	10.7 ±5.2	13.8 ±4.4
Day 4				
Trial time (secs - mean of 3 trials)	18.6± 9.1	30.4±13.8	20.7± 7.5	36.4**± 21.6
Number of failed trials (90 secs)	0.0±0.0	0.3± 0.5	0.0±0.0	0.4 ±0.7
Percentage with at least 1 failed trial (90	0.0	33.3	0.0	27.3

TABLE 23. Watermaze performance data (mean ±SD) in male F_1 rats [a]

Parameter	Dose (mg/kg/day)			
	0	2	10	50
secs) Sector entries (mean of 3 trials)	6.9±2.9	10.8±5.2	7.4 ±.6	9.6± 4.1
PND 61				
Day 1 Trial time (secs - mean of 3 trials) Number of failed trials (90 secs) Percentage with at least 1 failed trial (90 secs) Sector entries (mean of 3 trials)	66.0± 10.5 1.6± 0.7 91.7 17.4±3.4	63.8±12.9 1.6 ±0.7 91.7 15.3±3.6	72.9±15.7 1.8± 0.9 91.7 18.6± 4.5	66.3± 19.1 1.7±0.8 90.9 16.9 5.9
Day 2 Trial time (secs - mean of 3 trials) Number of failed trials (90 secs) Percentage with at least 1 failed trial (90 secs) Sector entries (mean of 3 trials)	36.6± 23.5 0.5 0.9 33.3 11.0 5.5	34.6±19.7 0.5 0.7 41.7 10.3 5.0	43.7±21.2 0.7 0.9 50.0 12.4 4.2	37.2± 21.4 0.3 0.5 27.3 11.6 6.3
Day 3 Trial time (secs - mean of 3 trials) Number of failed trials (90 secs) Percentage with at least 1 failed trial (90 secs) Sector entries (mean of 3 trials)	19.3±22.3 0.3±0.6 16.7 6.4 ±5.9	28.7*±18.9 0.2± 0.6 8.3 8.4± 3.5	25.8*±16.9 0.2 ±0.4 16.7 7.4± 3.2	29.4* ±18.8 0.3±0.5 27.3 8.1 ±4.3
Day 4 Trial time (secs - mean of 3 trials) Number of failed trials (90 secs) Percentage with at least 1 failed trial (90 secs) Sector entries (mean of 3 trials)	14.5± 13.4 0.2±0.4 16.7 5.2 ±3.5	28.5*± 14.2 0.2± 0.4 16.7 8.5±3.9	18.3*± 9.4 0.1±0.3 8.3 6.1 ±3.1	19.2*± 10.0 0.0± 0.0 0.0 6.3 ±2.9

[a]Data obtained from pages 193 and 195, MRID 46534401.
PND = postnatal day
* Significantly different from control group, $p<0.05$.
** Significantly different from control group, $p<0.01$.

TABLE 24. Watermaze performance data (mean ±SD) in female F_1 rats [a]

Parameter	Dose (mg/kg/day)			
	0	2	10	50
	PND 23/24			
Day 1 Trial time (secs - mean of 3 trials) Number of failed trials (90 secs) Percentage with at least 1 failed trial (90 secs) Sector entries (mean of 3 trials)	59.6 ±16.8 1.2 ±0.8 75.0 17.8 ±5.5	58.8 ±5.9 1.0± 0.7 75.0 17.2 ±3.1	68.6±20.5 1.6±1.1 81.8 17.2±4.5	57.3±21.0 0.9± 1.0 60.0 15.4± 4.1
Day 2				

TABLE 24. Watermaze performance data (mean ±SD) in female F_1 rats [a]

Parameter	Dose (mg/kg/day) 0	2	10	50
Trial time (secs - mean of 3 trials)	53.5 ±20.6	35.9±19.1	43.6± 22.8	33.8 ±18.6
Number of failed trials (90 secs)	0.9±0.9	0.3 ±0.5	0.6±0.9	0.3*± 0.7
Percentage with at least 1 failed trial (90 secs)	66.7	25.0	45.5	20.0
Sector entries (mean of 3 trials)	15.5±6.3	11.2± 5.1	13.0± 5.6	8.7**± 3.0
Day 3				
Trial time (secs - mean of 3 trials)	35.8± 15.4	30.1± 10.5	35.5 ±13.7	32.3±15.9
Number of failed trials (90 secs)	0.3± 0.5	0.2±0.4	0.3±0.5	0.3± 0.5
Percentage with at least 1 failed trial (90 secs)	33.3	16.7	27.3	30.0
Sector entries (mean of 3 trials)	10.3 ±4.4	9.8±2.5	10.7±3.2	8.8 ±3.8
Day 4				
Trial time (secs - mean of 3 trials)	25.2± 19.8	27.3±14.7	32.1±15.3	16.1± 10.5
Number of failed trials (90 secs)	0.3±0.6	0.2 ±.4	0.3±0.6	0.0±0.0
Percentage with at least 1 failed trial (90 secs)	16.7	16.7	18.2	0.0
Sector entries (mean of 3 trials)	8.5± 4.4	8.8 ±3.9	9.6 ±5.2	5.7± 2.7
PND 61				
Day 1				
Trial time (secs - mean of 3 trials)	69.5 ±15.0	60.0 ±18.3	68.2 ±19.2	64.0 ±16.1
Number of failed trials (90 secs)	1.3±1.0	1.5 ±.8	1.5 ±.2	1.0 ±0.6
Percentage with at least 1 failed trial (90 secs)	83.3	100.0	72.7	81.8
Sector entries (mean of 3 trials)	17.5 ±3.2	17.0 ±6.6	17.0±3.9	15.4± 4.8
Day 2				
Trial time (secs - mean of 3 trials)	43.4 ±11.5	39.3±15.7	51.2± 21.6	38.6± 20.4
Number of failed trials (90 secs)	0.4 ±0.5	0.3± 0.7	0.9±1.0	0.5± 0.7
Percentage with at least 1 failed trial (90 secs)	41.7	25.0	54.5	45.5
Sector entries (mean of 3 trials)	13.6±3.3	12.0±4.5	13.2 ±4.4	10.5 ±4.0
Day 3				
Trial time (secs - mean of 3 trials)	31.0 ±17.6	35.9 ±22.6	27.1± 10.0	29.7 ±8.0
Number of failed trials (90 secs)	0.3 ±0.5	0.5±0.9	0.1±0.3	0.2±0.6
Percentage with at least 1 failed trial (90 secs)	33.3	33.3	9.1	9.1
Sector entries (mean of 3 trials)	9.3 ±4.6	9.2±3.9	7.6 ±2.3	8.3± 3.7
Day 4				
Trial time (secs - mean of 3 trials)	27.9± 15.5	20.1± 9.5	22.6± 11.9	19.0±15.9
Number of failed trials (90 secs)	0.0 ±0.0	0.0±0.0	0.1 ±0.3	0.2± 0.6
Percentage with at least 1 failed trial (90 secs)	0.0	0.0	9.1	9.1
Sector entries (mean of 3 trials)	8.4±4.6	6.1 ±2.6	6.3± 3.4	5.9± 3.2

[a]Data obtained from pages 194 and 196, MRID 46534401.
PND = postnatal day
* Significantly different from control group, $p<0.05$.
** Significantly different from control group, $p<0.01$.

5. Postmortem results:

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a. ***Brain weight:*** Mean perfused brain weight, length and width data are presented in Table 25.Absolute brain weight for the high dose males on PND 21 was considered to have a treatment related decrease (6.1%, $p < 0.05$). Brain width in males at 50 mg/kg/day was significantly decreased (3-4%) on PNDs 21 and 66. On PND 21, the mean value at this dose was influenced by one male (#0203) which had very low (11.4 mm) brain width; exclusion of this animal resulted in a mean value that was not statistically significant. Non-perfused brain weight was significantly decreased in males (3-5%) and females (4-5%) at 10 and 50 mg/kg/day on PND 66 (Table 26).

TABLE 25. Mean (±SD) Perfused Brain Weight and Measurement Data in F_1 rats [a]

Parameter	Dose (mg/kg/day)			
	0	2	10	50
PND 21 - Perfused Males				
Brain weight (g)	1.47 ± 0.09	1.46 ±0.11	1.45 ±0.08	1.38 ±0.11*(a)(↓6.1%)
Brain length (mm)	17.9 ±0.6	18.4 ±0.6	18.1± 0.4	18.2± 0.6
Brain width (mm)	14.8 ±0.4	14.7 ±0.4	14.9 ±1.6	14.2* ±1.0 ↓(4%)
PND 66 - Perfused Males				
Brain weight (g)	2.07 ± 0.06	2.06± 0.13	2.00 ±0.10	1.98± 0.15
Brain length (mm)	21.3 ±0.5	21.2±0.5	21.2± 0.6	21.5± 0.4
Brain width (mm)	15.6±0.3	15.4±0.3	15.3± 0.4	15.2**± 0.5 ↓(3%)
PND 21 - Perfused Females				
Brain weight (g)	1.44± 0.08	1.43 ±0.12	1.39 ±0.07	1.39 ±0.08
Brain length (mm)	17.8 ±0.6	18.0±0.6	18.1±0.5	18.1±0.6
Brain width (mm)	14.5±0.5	14.3±0.4	14.4± 0.3	14.3 ±0.5
PND 66 - Perfused Females				
Brain weight (g)	1.97 ±0.12	1.90 ±0.08	1.92 ±.07	1.84± 0.10
Brain length (mm)	20.5 ±0.6	20.7± 0.7	20.9± 0.6	20.8 ±0.7
Brain width (mm)	15.1±0.4	14.9± 0.4	14.8 ±0.4	14.8± 0.5

[a] Data obtained from pages 217-218 and 221-222, MRID 46534401.
N= 24 on PND 21; 11-13 on PND 61
* Significantly different from control group, $p<0.05$.
** Significantly different from control group, $p<0.01$.
*(a) $p < 0.02$ based on unadjusted weight. Refer to MRID 470118301 page 11.

TABLE 26. Mean (±SD) Non-perfused Brain Weight Data (g) in F_1 rats on PND 66[a]				
Parameter	Dose (mg/kg/day)			
	0	2	10	50
Males	2.11± 0.07	2.08 ±0.10	2.04**± 0.09 (↓3%)	2.01**± 0.11 (↓5%)
Females	1.96±0.07	1.93± 0.10	1.89**± 0.09 (↓4%)	1.87**±0.11 (↓5%)

[a] Data obtained from page 220, MRID 46534401.
N= 42-49
** Significantly different from control group, p<0.01.

b. **Macroscopic examination:** Examination of offspring that died before the scheduled necropsy showed that eight animals in the 10 mg/kg/day group had a ruptured esophagus resulting from a technical dosing error. No treatment-related macroscopic lesions were observed in selected neuropathology animals at PNDs 21 and 66 or in unselected animals at the PND 28 necropsy.

c. **Neuropathology**:

1) ***Microscopic examination:*** No treatment-related lesions were observed at the PNDs 21 and 66 necropsy. Minimal or slight degeneration of some peripheral nerves was observed in both the control and treated groups. One male of the 11 examined in the high dose group had an incident of "vacuolation of the grey matter – molecular layer" a possible condition that may result from the impurity #5 when examined on day 66. Since only one animal was affected on day 66 and none were affected on day 21 and the condition due to impurity #5 is known to be reversible, this incident is not considered to be related to treatment. .

2) **Brain Morphometry:** Morphometric measurements are presented in Table 27. No treatment-related effects were observed.

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TABLE 27. Mean (±SD) morphometric measurements (mm) in F_1 rats[a]		
Parameter	Dose (mg/kg/day)	
	0	50
PND 21 - Males		
Neocortex	1.83±0.08	1.78 ±0.13
Hippocampus	1.82±0.12	1.80 ±0.13
Corpus Callosum	0.21±0.07	0.21± 0.02
Cerebellum	0.81±0.09	0.84 ±0.06
PND 66 - Males		
Neocortex	1.91± 0.10	1.83± 0.11
Hippocampus	2.08 ±0.08	1.93± 0.17
Corpus Callosum	0.29 ±0.04	0.26± 0.06
Cerebellum	0.86± 0.06	0.87 ±0.09
PND 21 - Females		
Neocortex	1.71 ±0.08	1.74 ±0.10
Hippocampus	1.82 ±0.11	1.75± 0.11
Corpus Callosum	0.18± 0.04	0.23± 0.06
Cerebellum	0.83± 0.09	0.83± 0.09
PND 66 - Females		
Neocortex	1.85±0.10	1.79± 0.05
Hippocampus	1.99±0.14	1.88± 0.09
Corpus Callosum	0.29 ±0.03	0.25± 0.03
Cerebellum	0.85 ±0.07	0.84± 0.06

[a] Data were obtained from pages 219 and 223, MRID 46534401.
N=9-10

III. DISCUSSION and CONCLUSIONS:

INVESTIGATORS CONCLUSIONS: The study author concluded that the maternal LOAEL was 10 mg/kg/day based on reduced body weight gain. The maternal NOAEL was 2 mg/kg/day. The offspring LOAEL was 10 mg/kg/day based on lower PND 1 body weight and body weight gain. The offspring NOAEL was 2 mg/kg/day.

B. **REVIEWER (contractor) COMMENTS:** Administration of Fluazinam at dosages up to 50 mg/kg/day by gavage resulted in no deaths or clinical signs of toxicity in maternal animals prior to the scheduled sacrifices. Mean body weight during gestation and lactation was not affected by treatment. Mean body weight *gain* was significantly decreased at 10 and 50 mg/kg/day during gestation but not during lactation. Food consumption (g/rat/day) during gestation was comparable to controls but was significantly decreased at 10 and 50 mg/kg/day during lactation. Mean body weight and body weight gain post weaning (days 28-35) were not affected by treatment. Reproductive parameters were not affected by treatment. The weight *gain* and food consumption decreases that occurs in the absence of absolute weight differences for the dams are not

considered to be of sufficient magnitude to be included as a true toxic response. Behavioral assessments, including FOB, motor activity, auditory startle reflex habituation and learning and memory, were not affected by treatment. No treatment-related changes were observed at either the LD 21 or PPD 66 necropsy.

At 50 mg/kg/day, the number of mean implantations and litter size (total and live) on Day 1 and Day 4 (pre-cull) was non-significantly decreased. These decreases are not considered toxicologically significant due to the lack of statistical significance and the low magnitude of difference from the control group. Offspring survival after culling in this group was comparable to the control group. At 10 mg/kg/day, a single incidence of total litter loss occurred on PND 3. Live litter size was significantly decreased during PNDs 11-21 in the 10 mg/kg/day group. Many of the deaths in this group were the result of a technical dosing error and were not treatment-related. The sex ratio in treated groups was comparable to the control group for all days on which it was calculated. Dark and/or distended abdomen was observed in males and females at 50 mg/kg/day. Although the number of offspring affected was small, similar findings were observed at equal and higher doses in the preliminary study and therefore, the signs are considered treatment-related. However at 10 mg/kg/day, these signs were found in animals that had a ruptured esophagus due to dosing error and are considered unrelated to treatment with the test article. **The LOAEL for maternal effects of Fluazinam was not established. The maternal NOAEL was not established.**

Offspring parameters. **Mean body weight and body weight gain** were significantly decreased in male and female offspring at 10 and 50 mg/kg/day prior to weaning. The weight difference persisted through the post-weaning period (PNDs 28-63). However, body weight gain in the treated groups was essentially comparable to the control group in the post weaning period. The mean age of **completion of balano-preputial separation** was significantly delayed in males at 10 (+1.2 days) and 50 (+3.1 days) mg/kg/day. Mean body weight at attainment was comparable to the control group. The delay is considered treatment-related and toxicologically significant.

The apparent differences observed during the FOB in treated groups are not considered treatment-related since there was no statistical significance or dose response. The only possible treatment-related changes were on PND 21 when decreased activity count in males at 10 and 50 mg/kg/day and increased number of males and females with one or both pupils failing to dilate during the pupil reflex testing were observed in the high dose group. Forelimb grip strength and hindlimb grip strength were significantly lower in females at 50 mg/kg/day on PNDs 21 and 35, respectively. Although these findings were not consistent, the reduced grip strength *may have* been due to lower body weight by these animals. The **peak amplitude in the auditory startle response** was affected in the high dose males on days 23/24. No treatment-related effects were observed on motor activity or and learning and memory. Statistically significant changes during the interval testing were sporadic and not dose-related. **Brain weight** in high dose males on PND 21 was decreased 6.1% and the width of the perfused brains of males at 50 mg/kg/day on PNDs 21 and 66 were significantly decreased. There was, however, no effect on brain length in males and females. Non-perfused brain weights in males and females at 10 and 50 mg/kg/day were significantly decreased on PND 66. No treatment-related macroscopic or microscopic findings were observed.

The offspring LOAEL is 10 mg/kg/day based on decreased body weight and body weight gain and delay in completion of balano-preputial separation. The offspring NOAEL is 2 mg/kg/day.

C. **STUDY DEFICIENCIES:** The study report is inconsistent in reporting the treatment duration for both parental females and offspring. Page 17 states that females were treated from Day 6 after mating to Day 20 of lactation and that offspring were dosed from Day 7 of age to Day 20 or 21 of age. Page 20 states that females were dosed from Day 6 after mating to Day 20 or 21 of lactation. Page 28 indicates that offspring were treated from Day 7 of age to Day 21 of age (inclusive). This apparent inconsistency does not affect the interpretation of the study.

In response to HED's request the registrant provided the range finding study (2005, MRID 46534401) to demonstrate that at 100 mg/kg/day and above there were pups deaths. Thus, although 50 mg/kg/day, the highest dose tested in this study, did not elicit effects, 50 mg/kg/day is considered acceptable as the high dose for this study since dosing at higher doses would result in pup death. In addition to the range finding study, the registrant submitted revised tables (2006, MRID No.: 47018301) to show the statistical notations for acoustic startle response unadjusted for body weight data. The peak amplitude for the males at PND 23/24 was determined to be affected. Tables 19 and 21 of this DER were changed from the original review to include the statistical notations based on the unadjusted data. The unadjusted brain weight from perfused animals in the PND 21 high dose males was determined to be statistically and toxicologically significant as indicated by a 6.1% decrease. Table 25 of this DER was changed from the original review to include the statistical notations on the unadjusted brain weight data.