US EPA ARCHIVE DOCUMENT

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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

October 3, 2005

MEMORANDUM

Subject: EPA Id No.: 083601. Triphenyltin Hydroxide: Review of the developmental neurotoxicity study (2003, MRID No.: 46055701) in rats.

TXR # 0052163
DP Barcode No.: D294568
PC Code: 083601

From: John Doherty
ReRegistration Branch III
Health Effects Division 7509C

To: Susan Bartow
Special Review and ReRegistration Division 7507C

Through: Catherine Eiden
Branch Chief
ReRegistration Branch III
Health Effects Division 7509C

Conclusion:

The developmental neurotoxicity study (2003, MRID No.: 46055701) with triphenyltin hydroxide (TPTH) has been reviewed by ReRegistration Branch III (RRBIII) and classified as Acceptable/Non-Guideline. The Non-Guideline status is currently being applied to all developmental neurotoxicity (DNT) studies pending review of the positive control data bases. The study with TPTH is further identified together with the Executive Summary in the following table. A copy of the DER is attached.

NOV 0 9 2005

Table. Study reviewed.

Study Identification	Executive Summary
870.6300. Developmental Neurotoxicity - Rats Huntingdon Life Sciences, Study No.: LDA/038, July 25, 2003. MRID No.: 46055701.	In a developmental neurotoxicity study (2003, MRID 46055701), Fentin hydroxide (TPTH; 97.5% a.i., Lot/Batch #: ZVRAM.928K) in corn oil was administered to pregnant CD (Crl:CD®[SD]BR IGS) rats (24/dose) by gavage at dose levels of 0, 1, 2.5, or 5 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20. Groups of 10 dams/dose were subjected to a functional observational battery (FOB) during gestation and lactation. On postnatal day (PND) 4, litters were standardized to 4 pups/sex pups/litter, and 1 pup/litter/group were allocated to subsets for assessment of FOB and motor activity, auditory startle response, learning and memory evaluation, and neuropathological examination. *Maternal Toxicity.* A single dam in the 2.5 mg/kg/day dose group was sacrificed on GD day 18 due to poor condition (pallor, piloerection, red discharge from the vagina and low body temperature). Six high dose dams were sacrificed with similar poor clinical conditions between GDs 21 to 23. Three high dose dams showed total litter death *in utero*, while some of the other three had obvious problems with parturition. There were 24, 24, 23 and 17 dams with viable litters for the control, low, mid and high dose groups. During the FOB assessment, the high dose group was judged to have different grades for ease of handling (reactivity, reduced rearing and activity levels). Body weight gains were decreased (17%) in the high dose group during gestation and increased (138% for days 1 - 21) during lactation but food consumption generally decreased (up to 22%.) during either gestation or lactation. There were 23, 23, 22 and 16 litters for the control, 1, 2.5 and 5 mg/kg/day dose groups, respectively that provided pups for the developmental aspects of this study. **The maternal LOAEL is 2.5 mg/kg/day, based on clinical condition (requiring sacrifice) in one animal with conditions similar to dams at the next higher dose. The maternal NOAEL is 1 mg/kg/day.** *Developmental Toxicity.* Starting at about day 49 and persisting to termination, the low (4.4 to 5.5% for males, and 4.7 to 5% for females, $p < 0.05$ of 0.01) and mid (4.8 to 6.5% for males and 3.7% to 4.6% for females, $p < 0.05$ or 0.01) dose group body weights were decreased. Mean body weight gain over the period of days 28 to 65 were also decreased for the low (6%, both sexes, $p < 0.01$) and mid (6% females and 7% males, both $p < 0.01$) dose groups. The mean litter size was similar at birth for all groups but mean pup weight at birth was slightly lower (19% for males and 16% for females, both $p < 0.05$) in the high dose group. During lactation, the high dose group pups reached 14 to 15% lower body weights and weight gain during lactation was reduced (15% for males and 20% for females for days 1 to 7). The high dose group maintained a 7 to 10% differences in post weaning body weight until day 65 accompanied with a decrease of 11% in body gain over this period. Treatment had no adverse effects on survival, clinical signs, food consumption, developmental landmarks, motor activity, auditory startle response, learning and memory, brain weights, brain morphology or neuropathology at any dose. **The offspring LOAEL is 5 mg/kg/day based on decreases in body weight and body weight gain. The offspring NOAEL is 2.5 mg/kg/day.** Note: The apparent differences in body weight in the low and mid dose group were no included in the LOAEL because of a lack of dose response and the were not considered definitely related to treatment. This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

DATA EVALUATION RECORD

FENTIN HYDROXIDE (TPTH)

Study Type: §83-6; Developmental Neurotoxicity Study in Rats

Work Assignment No. 1-01-20; (MRID 46055701)

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

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Michael E. Viana, Ph.D.

Signature: Michael E Viana
Date: 4/30/04

Secondary Reviewer:
David A. McEwen, B.S.

Signature: David A. McEwen
Date: 4/30/04

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 4/30/04

Quality Assurance:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 4/30/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John Doherty **Signature:** [signature]
Toxicology Branch, Health Effects Division (7509C) **Date** Sept. 29, 2005
EPA Work Assignment Manager: Ghazi Dannan, Ph.D. **Signature:** [signature]
Registration Action Branch 3, Health Effects Division (7509C) **Date** 10/3/05
Template version 11/01

DATA EVALUATION RECORD

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

PC CODE: 083601 **DP BARCODE:** D294568
TXR#: 0052163 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Fentin Hydroxide (TPTH); 97.5% a.i.

SYNONYMS: Triphenyltin hydroxide; AE F029664

CITATION: Myers, D., Bottomley, A., Renaut, S., *et al.* (2003) TPTH: developmental neurotoxicity study in the CD rat by oral administration. Huntingdon Life Sciences, Ltd., Alconbury, Huntingdon, Cambridgeshire, England. Laboratory Project Id. No.: LDA/038/032055, July 25, 2003. MRID 46055701. Unpublished.

SPONSOR: TPTH Task Force, c/o Landis International, Inc., 3185 Madison Highway, Valdosta, Ga

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2003, MRID 46055701), Fentin hydroxide (TPTH; 97.5% a.i., Lot/Batch #: ZVRAM.928K) in corn oil was administered to pregnant CD (Crl:CD®[SD]BR IGS) rats (24/dose) by gavage at dose levels of 0, 1, 2.5, or 5 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20. Groups of 10 dams/dose were subjected to a functional observational battery (FOB) during gestation and lactation. On postnatal day (PND) 4, litters were standardized to 4 pups/sex pups/litter, and 1 pup/litter/group were allocated to subsets for assessment of FOB and motor activity, auditory startle response, learning and memory evaluation, and neuropathological examination.
Maternal Toxicity. A single dam in the 2.5 mg/kg/day dose group was sacrificed on GD day 18 due to poor condition (pallor, piloerection, red discharge from the vagina and low body temperature). Six high dose dams were sacrificed with similar poor clinical conditions between GDs 21 to 23. Three high dose dams showed total litter death *in utero*, while some of the other three had obvious problems with parturition. There were 24, 24, 23 and 17 dams with viable litters for the control, low, mid and high dose groups. During the FOB assessment, the high dose group was judged to have different grades for ease of handling (reactivity, reduced rearing and activity levels). Body weight gains were decreased (17%) in the high dose group during gestation and increased (138% for days 1 - 21) during lactation but food consumption generally decreased (up to 22%.) during either gestation or lactation. There were 23, 23, 22 and 16 litters for the control, 1, 2.5 and 5 mg/kg/day dose groups, respectively that provided pups for the

developmental aspects of this study. **The maternal LOAEL is 2.5 mg/kg/day, based on clinical condition (requiring sacrifice) in one animal with conditions similar to dams at the next higher dose. The maternal NOAEL is 1 mg/kg/day.**
Developmental Toxicity. Starting at about day 49 and persisting to termination, the low (4.4 to 5.5% for males, and 4.7 to 5% for females, $p < 0.05$ of 0.01) and mid (4.8 to 6.5% for males and 3.7% to 4.6% for females, $p < 0.05$ or 0.01) dose group body weights were decreased. Mean body weight gain over the period of days 28 to 65 were also decreased for the low (6%, both sexes, $p < 0.01$) and mid (6% females and 7% males, both $p < 0.01$) dose groups. The mean litter size was similar at birth for all groups but mean pup weight at birth was slightly lower (↓9% for males and ↓6% for females, both $p < 0.05$) in the high dose group. During lactation, the high dose group pups reached 14 to 15% lower body weights and weight gain during lactation was reduced (15% for males and 20% for females for days 1 to 7). The high dose group maintained a 7 to 10% differences in post weaning body weight until day 65 accompanied with a decrease of 11% in body gain over this period. Treatment had no adverse effects on survival, clinical signs, food consumption, developmental landmarks, motor activity, auditory startle response, learning and memory, brain weights, brain morphology or neuropathology at any dose. **The offspring LOAEL is 5 mg/kg/day based on decreases in body weight and body weight gain. The offspring NOAEL is 2.5 mg/kg/day.** Note: The apparent differences in body weight in the low and mid dose group were no included in the LOAEL because of a lack of dose response and the were not considered definitely related to treatment.

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

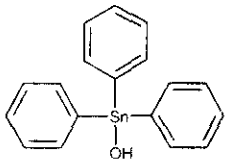
COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Fentin Hydroxide (TPTH)

Description: White powder
Lot/Batch #: ZVRAM.928K
Purity: 97.5% a.i.
Compound Stability: Stable for at least 2 days at ambient temperature or 15 days refrigerated
CAS # of TGAI: 76-87-9
Structure



2. Vehicle and/or positive control: Corn oil

3. Test animals (P):

Species:	Rat	
Strain:	CD (Crl:CD®(SD)BR IGS)	
Age at study initiation:	10-11 wks	
Wt. at study initiation:	225-334 g	
Source:	Charles River UK, Ltd. (Margate, Kent, England)	
Housing:	Acclimation: TR18 stainless steel cages with stainless steel grid floors, up to 4/cage Mating and Gestation (up to Day 17): RB3 high density polypropylene cages with stainless steel grid floors, 1 male/1 female or 1 female Littering (Day 17 of gestation to Days 14-18 of lactation): RB3 high density polypropylene cages with solid floors, 1 female with litter Lactation (Days 14-17 to weaning): TR18 stainless steel cages with stainless steel grid floors, 1 female with litter F1 generation from weaning: TR18 stainless steel cages with stainless steel grid floors, up to 4 littermates	
Diet:	UAR VRF1 Certified pelleted rodent diet (Usine d'Alimentation Rationelle, France), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	15/hr
	Photoperiod:	12hrs light/12 hrs dark
Acclimation period:	At least 1 week	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: 06/19/02 End: 09/18/02

2. Study schedule: The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day (GD) 6 through lactation day (LD) 20. Pups were weaned on postnatal day (PND) 21, after which time maternal animals were killed. F1 pups remained on study until PND 65 (study termination).

3. Mating procedure: Females were paired (1:1) with stock males of the same strain over an 11-day period. Mating was determined by daily observations of ejected copulatory plugs and/or the presence of sperm in a vaginal smear. The day on which sperm or at least 3 copulatory plugs were observed was designated GD 0.

4. Animal Assignment: Mated females were sequentially assigned to dose groups as indicated in Table 1. Dams were assigned to functional observation testing as shown. Offspring were assigned to testing subgroups at the time of litter standardization on postnatal day 4 (Table 1).

Table 1. Study design[a]

Experimental Parameter	Dose (mg/kg/day)			
	0	1	2.5	5
Maternal Animals				
No. of maternal animals assigned	24	24	24	24
FOB (GD 12,18; LD 4, 11, 21)	10	10	10	10
Offspring [M/F]				
Detailed clinical/FOB (PND 4, 11, 21, 335, 45, 60)	12/11	12/11	12/10	12/10
Motor activity (PND 13, 17, 22, 59)	12/11	12/11	12/10	12/10
Auditory startle habituation (PND 23/24, 60/61)	11/12	11/12	10/11	11/10
Auditory startle pre-pulse inhibition (PND 23/24, 60/61)	12/11	12/11	12/10	8/7
Learning and memory (PND 23/24)	11/12	11/12	10/12	10/11
Learning and memory (PND 61)	12/11	12/11	12/10	10/10
Brain weight, length, and width PND 21 PND 65	 10/13 10/10	 11/12 10/10	 10/12 10/10	 10/10 10/10
Brain weight only PND 65[b]	 48/46	 48/47	 46/42	 32/32
Brain morphology PND 21 PND 65	 10/10 10/10	 NE NE	 NE NE	 10/10 10/10
Neuropathology PND 21 PND 65	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex

a Data were obtained from pages 27 and 123-129 of the study report.
b Remaining animals not selected for neuropathology on Day 65.
NE Not examined

One pup/sex/litter was allocated to each behavioral test from the 0, 1, and 2.5 mg/kg groups. However, in the 5 mg/kg group, offspring were derived from a total of 15 litters due to maternal mortality, compared with a minimum of 22 litters in the other study groups. The same individual animals were evaluated for FOB and motor activity testing at all scheduled time

points. Different animals were used for cognitive (learning and memory) assessments at the 2 time points.

5. Dose selection rationale: Dose levels were chosen based on the results of a preliminary study in pregnant rats (Huntingdon Life Sciences Report No. LDA 037/013576) in which administration of TPTH at 5 mg/kg/day was associated with transient, slight decreases in maternal body weight gain and food consumption during gestation, and slight decreases in implantation number and litter size (data not provided). A single death was considered unlikely to be related to treatment.

6. Dosage preparation, administration, and analysis: Test substance formulations were prepared weekly. The highest concentration was prepared first by grinding an appropriate amount of test substance in a mortar and adding small volumes of corn oil, forming a smooth pourable suspension. The suspension was transferred to a graduated cylinder and brought up to final volume with corn oil. This suspension was poured into a beaker and mixed with a magnetic stirrer until a homogeneous suspension was visible. The lower concentrations were then prepared from this formulation by serial dilution with corn oil. Formulations were stored refrigerated. All doses were administered once daily to maternal animals by gavage, on GD 6 through LD 20, in a volume of 5 mL/kg of body weight/day. Dosing was based on the most recent body weight determination up to and including GD 17; thereafter, the dosing volume remained constant until LD 1. From LD 1 to LD 20, dosing was again based on the most recent body weight determination. Control animals received vehicle at the same volume dosage as treated groups. Prior to the start of the study, homogeneity and stability of the test substance in vehicle at concentrations of 0.002 and 2.0 mg/mL were confirmed for a period of 2 days at room temperature or 15 days refrigerated (Huntingdon Life Sciences Report LDA 037/013576; data not provided). During the study, samples of all formulations used during Week 1 of gestation and Week 1 of lactation were analyzed for concentration.

Results:

Concentration Analysis (% of nominal): 86-104%

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: Twice daily checks for mortality or moribundity were conducted for maternal animals. Gross observations of the dams were conducted daily, prior to treatment. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration. Additional observations were made on each treatment day according to the following schedule: (i) pre-dosing; (ii) on return to home cage; (iii) at the end of group dosing; (iv) 1-2 hours after dosing of all groups; and (v) as late as possible in the working day. Each animal was given a full physical examination on GD 0, 6, 13, and 20, and dams that littered were examined on LD 1, 7, 14, and 21. Individual maternal body weight data were recorded on GD 0, 3, 6, 10,

14, 17, and 20, daily until parturition, and on LD 1, 4, 7, 11, 14, 17, and 21. Food consumption was recorded on GD 0-2, 3-5, 6-9, 10-13, 14-16, and 17-19, and on LD 1-3, 4-6, 7-10, 11-13, 14-16, and 17-20.

Ten dams per group were subjected to a modified functional observational battery (FOB) outside the home cage prior to dosing on GD 12 and 18, and on LD 4, 11, and 21. It was not stated that the technicians were blind to the dose group of the dam being tested. The functional observations included, but were not limited to, the following:

FUNCTIONAL OBSERVATIONS	
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 exophthalmos, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

b. Offspring

1) Litter observations: The day of completion of parturition was designated as PND 0. Live pups were counted, sexed, and weighed individually for each litter on PND 1. Pups were examined cage-side daily for mortality, morbidity, and clinical signs of toxicity through PND 21. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. Pups were also weighed on PND 4, 7, 11, 14, 17, 21, and 28, and sexes were reconfirmed on PND 4 and 21.

On PND 4, litters were standardized by random selection to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). On PND 29. Any pups not allocated to further investigations were killed.

2) Developmental landmarks: Beginning on PND 32, male offspring were examined daily for balanopreputial separation. Body weight was recorded on the day of start and completion of separation. Female offspring were examined daily beginning on PND 28 for vaginal patency. The age of onset and body weight on the day of opening was recorded.

3) Postweaning observations: After weaning on PND 21, offspring were examined twice daily for mortality and morbidity. Each animal was subjected to a full physical examination weekly until termination. Individual offspring body weight data were recorded weekly.

4) Neurobehavioral evaluations:

i) Functional observational battery (FOB): On PND 4, 11, 21, 35, 45, and 60, 10-12 offspring/sex/group (one male or one female from each litter) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed. On PND 4, pups were placed in a clear perspex arena having a paper marked with concentric circles underneath it. The animal was placed in the center of the circles and was observed for 1 minute, during which time the following parameters were assessed: speed of righting; sections entered; maximum distance traveled; maximum pivoting angle relative to starting position; physical condition; lack of locomotor coordination; and other abnormal behaviors. On PND 11, animals were placed in the same arena, but the paper was marked with 9 rectangles of equal area. Animals were assessed for speed of righting, then were placed in the center of the arena for a 1-minute recording period, during which time the following parameters were assessed: number of sections entered; number of rearings; frequency of grooming; urination; physical condition; lack of locomotor coordination; and other abnormal behaviors. On PND 21, 35, 45, and 60, the following standard arena observations were recorded: palpebral closure; posture; gait; tremor; twitching; convulsions; activity; rearing; grooming; urination; and defecation. Additionally, on PND 35, 45, and 60, the following in-hand observations were recorded: removal from cage; salivation; lacrimation; piloerection; exophthalmos; reactivity to handling; and fur condition. Pupillary closure reflex was also measured on PND 35.

ii) Motor activity testing: Motor activity was evaluated on PND 13, 17, 21, and 59 in the same 10-12 rats/sex/dose used for FOB assessment. Animals were placed in a plastic cage and motor activity was monitored over a 1-hour period. The automated activity monitoring system (details not provided) collected data over ten 6-minute intervals using infra-red light sources and detectors set at 2 heights above the cage floor. Low beam detectors recorded ambulatory activity; high beam detectors recorded rearing. A raised insert was placed in the cage during PND 13 and 17 testing to ensure activity of the animals was being recorded by the low beam detectors. Eye opening was also recorded on PND 13.

iii) Auditory startle response habituation and pre-pulse inhibition of startle: Auditory startle response habituation and pre-pulse inhibition of startle testing was performed on 7-12 offspring/sex/dose on postnatal days 23/24 and 60/61, using an automated system (details not provided). For auditory startle response habituation, mean startle amplitudes were recorded during 5 consecutive blocks of 10 trials (50 trials total). The stimulus consisted of 40-millisecond bursts of white noise at approximately 105 dB against a background noise level of 70 dB, with inter-stimulus intervals of 12 seconds. Animals were acclimated to background noise for 5 minutes. For pre-pulse inhibition of startle, mean startle amplitudes were recorded for 10 trials with a pre-pulse of sound immediately preceding the startle stimulus, and for 10 trials without a pre-pulse. The pre-pulse consisted of a 50-millisecond burst of white noise at approximately 85 dB, followed by a 150-millisecond pause, then a 50-millisecond startle stimulus of white noise at approximately 118 dB. A total of 20 trials (10 of each) were presented in a pseudo-random order with inter-trial intervals of 10, 12, 14, or 16 seconds selected from a Latin square sequence.

iv) Learning and memory testing - Morris water maze: On PND 23/24 and approximately PND 61, learning and memory testing was performed in 10-12 offspring/sex/dose in a water-filled maze paradigm. A series of 3 trials were conducted on each of 4 consecutive days.

Different animals were used for testing on PND 23/24 and 61. The Morris maze consisted of a circular pools constructed of white plastic (90 cm diameter, 30 cm deep on PND 23/24; 140 cm diameter, 45 cm deep on PND 61). The maze was filled with water (temperature 29±3°C) made opaque using a non-toxic opacifier (Opacifier 621, manufacturer not provided). A 6 cm^2 platform was located at a fixed point in the pool, concealed approximately 1.5 cm below the surface. Three starting points were identified on the perimeter of the pool. A number of visual cues were placed on the walls of the pool; several cues were also present outside the pool to assist learning. On each day of testing, each animal received 3 consecutive trials. On the first trial, the rat was placed on the escape platform for 30 seconds prior to testing to optimize performance. 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 together 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 recorded.

2. Postmortem observations

a. Maternal animals: Maternal animals were sacrificed by CO_2 inhalation on LD 21 and subjected to a detailed macroscopic necropsy. The number of implantation sites was recorded, and the brain was removed, weighed, and fixed along with any abnormal tissues. Mammary tissue was examined and specimens retained from females whose litters died during early lactation. Carcasses were then discarded.

b. Offspring: The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 21 and 65. These animals were subjected to postmortem examinations as described below.

On PND 21, 10-13 pups/sex/group were killed by an overdose of barbiturate followed by perfusion fixation with glutaraldehyde and paraformaldehyde via the aorta, followed by immersion in fixative. Following perfusion, the animals were subjected to a macroscopic necropsy, and specimens of any abnormal tissues were preserved. The brain was transected from the spinal cord above the first cervical spinal nerve, and the length of the brain was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum. The width of the brain was measured at the widest part of the cerebral hemispheres. The brain was then weighed. The preserved brain and any abnormal tissue specimens were embedded in paraffin, blocked, sectioned, and stained with hematoxylin and eosin. Coronal sections of the olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, tectum, tegmentum, and medulla oblongata, and mid-sagittal sections of the cerebellum and pons from control and high-dose pups were examined by light microscopy. Morphometric measurements of the thickness of the neocortex, corpus callosum, hippocampus, and folia of the cerebellum (pyramis) were recorded. Animals not allocated to histopathology were necropsied, the brain was removed, weighed, and fixed in 10% neutral buffered formalin, and any abnormal tissues were sampled and preserved.

On PND 65, 10 animals/sex/group were killed, perfused, and the brain was processed as described above. The following central and peripheral nervous tissues (X) were dissected, fixed,

embedded in paraffin (CNS tissues) or resin (PNS tissues), blocked, sectioned, and stained with hematoxylin and eosin (CNS tissues) or toluidine blue (PNS tissues): brain, dorsal root fibers and ganglia (cervical and lumbar), eyes, optic nerves, sciatic nerves, gastrocnemius, spinal cord, tibial nerves, ventral root fibers, and any abnormalities. Histopathological evaluation was performed on tissues from males and females in the control and 5 mg/kg/day groups. Morphometric measurements of the thickness of the neocortex, corpus callosum, hippocampus, and folia of the cerebellum (pyramis) were recorded. Animals not allocated to histopathology were necropsied, the brain was removed, weighed, and fixed in 10% neutral buffered formalin, and any abnormal tissues were sampled and preserved.

The CHECKED (X) tissues were evaluated for adult offspring:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic Notch
X	Forebrain		
X	Cerebrum		**OTHER**
X	Hippocampus		Sural Nerve
X	Thalamus	X	Tibial Nerve
X	Hypothalamus		Peroneal Nerve
X	Tectum	X	Lumbar dorsal root ganglion
X	Tegmentum	X	Lumbar dorsal root fibers
X	Medulla Oblongata	X	Lumbar ventral root fibers
	SPINAL CORD	X	Cervical dorsal root ganglion
X	Cervical swelling (C3-C6)	X	Cervical dorsal root fibers
X	Lumbar swelling (L1-L4)	X	Cervical ventral root fibers
	OTHER		
X	Optic nerve		
X	Eyes (retina)		
X	Skeletal muscle		

D. DATA ANALYSIS

1. Statistical analyses: Tests for statistical significance were performed on the following parameters: gestation and lactation body weight change and food consumption, gestation index, litter size, pup body weight and body weight gain, body weight and body weight gain for pups selected for behavioral testing, balano-preputial separation data, maternal activity and rearing counts, PND 11, 21, 35, 45, and 60 activity and rearing counts, PND 13, 17, 22, and 59 motor activity, pre-pulse inhibition and habituation of startle response, Morris water maze trial times, sector counts, and number of failures, and brain morphometry data. If 75% of the data across all groups were the same value, a frequency analysis was applied. Treatment groups were compared using a Mantel test for trend in proportions and pairwise Fisher's Exact tests for each dose group against the controls. Bartlett's test for variance homogeneity was applied. If Bartlett's test was not significant ($p \leq 0.01$), parametric analysis was used. If the F1 test for monotonocity of dose-response was not significant ($p \leq 0.01$), Williams' test for monotonic trend

was applied; if the F1 test was significant, Dunnett's test was performed. If Bartlett's test was significant, logarithmic and square-root transformations of the data were tried. If Bartlett's test was still significant, non-parametric tests were performed. If the H1 test for monotonicity of dose-response was not significant ($p \leq 0.01$), Shirley's test for a monotonic trend was applied. Gestation length/index data was analyzed with the Cochran-Armitage test; brain morphometry data were analyzed with Student's t-tests.

2. Indices

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

Gestation index (%) = (# of live litters born/# pregnant) x 100

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

Post-implantation survival index = (total # of offspring born/total # of implantation sites) x 100

Live birth index = (# of live offspring at PND 1/total # of offspring born) x 100

Viability index = (# of live offspring at PND 4/# of live offspring at PND 1) x 100

Lactation index = (# of live offspring on Day of examination/# of live offspring on PND 4 after culling) x 100

3. Positive and historical control data: Positive control data were not submitted with this study; however, summaries of positive control data previously submitted to the Agency are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical and functional observations: Six 5 mg/kg females were killed between GD 21 and 23 due to poor clinical condition or parturition difficulties. Principal clinical signs included pallor, piloerection, red/brown discharge from the vagina, and low body temperature in some animals. All of these animals were pregnant, but 3 showed total litter death *in utero*. These deaths were considered to be related to treatment.

One 2.5 mg/kg female was killed on GD 18 due to poor clinical condition, after displaying pallor, piloerection, red discharge from the vagina, and low body temperature. Since this mortality occurred earlier than those at 5 mg/kg, it is considered incidental by the study author. However, since the clinical signs were similar to the animals dosed at 5 mg/kg/day and such reactions may be expected from TPTH, this animals condition is considered treatment related by the HED reviewer.

Among survivors, piloerection was observed in three 5 mg/kg females during parturition, on LD 1, and on LD 4, 5, 7, and 14. Red staining/discharge from the vagina was noted on GD 24-25, during parturition, and on LD 1. No other treatment-related clinical signs of toxicity were observed.

During the handling FOB observations, the 5 mg/kg dams were observed to have increased proportions of grade 2 (easy) vs grade 3 (some resistance or avoidance; slightly awkward) scores for removal from cage and/or reactivity to handling on GD 18, and LD 4, 11, and 21 (Table 2). During arena observations, the 5 mg/kg dams were also noted to have reduced activity and rearing counts (↓25-87%; not significant [NS]) on GD 18, and LD 4 and 11. On LD 21, activity and rearing counts were observed to be increased (↑8-34%; NS). No other FOB effects were noted.

No effects of treatment were observed on mortality, clinical signs of toxicity, or FOB effects at 1 or 2.5 mg/kg.

Table 2. Selected maternal functional observational battery parameters (# of observations/# of animals) in rats treated with TPTH.[a]

Parameter	Grade	Dose (mg/kg/day)			
		0	1	2.5	5
Gestation Day 18					
Removal from cage	2 (easy)	5/10	6/10	7/9	10/10
	3 (some resistance or avoidance)	5/10	4/10	2/9	0/10
Reactivity to handling	2 (easy)	1/10	2/10	2/9	5/10
	3 (slightly awkward)	9/10	8/10	7/9	5/10
Activity count	Not applicable	6.8	6.7	5.6 (↓18%)	5.1 (↓25)
Rearing count	Not applicable	2.4	2.2	2.1	1.8 (↓25)
Lactation Day 4					
Removal from cage	2 (easy)	8/10	9/10	9/9	6/6
	3 (some resistance or avoidance)	2/10	1/10	0/9	0/6
Reactivity to handling	2 (easy)	2/10	1/10	6/9	6/6
	3 (slightly awkward)	8/10	9/10	3/9	0/6
Activity count	Not applicable	4.8	7.3	4.1(↓15%)	2.3 (↓52)
Rearing count	Not applicable	1.5	2.2	2.0	1.0 (↓33)
Lactation Day 11					
Removal from cage	2 (easy)	8/10	10/10	7/9	6/6
	3 (some resistance or avoidance)	2/10	0/10	2/9	0/6
Reactivity to handling	2 (easy)	1/10	2/10	0/9	2/6
	3 (slightly awkward)	9/10	8/10	9/9	4/6
Activity count	Not applicable	8.4	8.2	6.0 (↓29%)	3.8* (↓55)
Rearing count	Not applicable	2.3	2.4	1.4 (↓39%)	0.3 (↓87)
Lactation Day 21					
Removal from cage	2 (easy)	5/10	6/10	5/9	5/6
	3 (some resistance or avoidance)	5/10	4/10	4/9	1/6
Reactivity to handling	2 (easy)	6/10	6/10	5/9	5/6
	3 (slightly awkward)	4/10	4/10	4/9	1/6
Activity count	Not applicable	9.7	12.7	7.8 (↓20%)	10.5 (↑8)
Rearing count	Not applicable	3.2	5.0	3.2	4.3 (↑34)

a Data were obtained from pages 72-81 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

* Significantly different from controls; $p \leq 0.05$

2. **<u>Body weight and food consumption</u>:** Selected group mean body weights, body weight gains, and food consumption values for pregnant or nursing dams are summarized in Tables 3a, b, and c. No effects of treatment were observed on body weights. Body weight gains were decreased ($p \leq 0.05$) by 17% in the 5 mg/kg females on GD 6-20. Body weight gains were then increased ($p \leq 0.01$) by 138% throughout lactation (LD 1-21). It was stated that this increase could have been due to reduced physiological demands on the females due to increased pup

mortality. There were no effects of treatment on body weight or body weight gains at 1 or 2.5 mg/kg.

Food consumption was decreased ($p \leq 0.05$) in the 5 mg/kg females on GD 6-9 and 17-19 (↓8-22%), and on LD 11-16 (↓11-12%). No effects of treatment were observed on food consumption at 1 or 2.5 mg/kg.

Table 3a. Mean (±SD) maternal body weights (g) in rats treated with TPTH.[a]

Observations	Dose (mg/kg/day)			
	0	1	2.5	5
Gestation (n=23-24)				
GD 0	272±27	271±28	283±25	279±19
GD 6	298±27	296±27	311±25	305±20
GD 14	340±29	344±28	358±26	350±21
GD 20	423±32	428±34	441±34	410±38
Lactation (n=16-23)				
LD 1	328±29	332±28	344±24	318±28
LD 7	344±31	348±30	360±23	346±22
LD 14	364±32	365±27	380±28	367±26
LD 21	352±25	360±28	375±28	375±26

a Data were obtained from page 82 of the study report.

Table 3b. Mean (±SD) maternal body weight gains (g) in rats treated with TPTH.[a]

Observations	Dose (mg/kg/day)			
	0	1	2.5	5
Gestation (n=23-24)				
GD 0-6	26±6	25±7	28±6	26±6
GD 6-14	42±6	48±6	46±6	44±9
GD 6-20	125±12	132±14	130±15	104±31* (↓17)
Lactation (n=16-23)				
LD 1-7	16±9	16±10	16±8	29±14** (↑81)
LD 1-14	36±13	33±11	36±13	50±16** (↑39)
LD 1-21	24±16	28±13	31±14	57±19** (↑138)

a Data were obtained from page 83 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.
* Significantly different from controls; p<0.05
** Significantly different from controls; p<0.01

Table 3c. Mean (±SD) maternal food consumption in rats treated with TPTH.[a]

Observations	Dose (mg/kg/day)			
	0	1	2.5	5
Gestation (n=23-24)				
GD 0-2	25±3	26±3	25±2	26±3
GD 6-9	25±2	25±2	24±2	23±3** (↓8)
GD 14-16	29±2	29±3	30±3	26±6
GD 17-19	27±2	28±3	28±4	21±7** (↓22)
Lactation (n=16-23)				
LD 1-3	35±5	36±7	37±11	33±14
LD 7-10	56±5	57±5	57±7	51±10
LD 11-13	68±8	66±7	64±11	60±11* (↓12)
LD 14-16	72±7	72±7	71±8	64±12** (↓11)
LD 17-20	79±14	82±10	81±13	79±17

a Data were obtained from page 84 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.
* Significantly different from controls; p<0.05
** Significantly different from controls; p<0.01

3. <u>Reproductive performance</u>: Reproductive performance is presented in Table 4. At 5 mg/kg, 6 females were killed for humane reasons and one was also killed at 2.5 mg/kg/day. One pregnant female in the high dose group had no young observed to be born. The duration of gestation for controls, 1 mg/kg, and 2.5 mg/kg females was 22-23 days. At 5 mg/kg, 2 females

demonstrated prolonged gestation (23.5 and 24.5 days) associated with subsequent elevated perinatal mortality. Since other females in this dose group displayed clinical signs of toxicity and morbidity and the distribution of gestation lengths was different ($p \leq 0.05$) from controls, this prolongation of gestation is considered treatment-related. Also at 5 mg/kg, the gestation index was decreased ($p \leq 0.001$), but this was primarily due to mortality at this dose level.

Table 4. Reproductive performance[a]

Observation	Dose (mg/kg/day)			
	0	1	2.5	5
Number mated	24	24	24	24
Number of litters	24	24	23	17
Intercurrent deaths	0	0	1	6
Pregnant - no young observed to be born	0	0	0	1
Gestation Day (Historical control in %)	Number of dams/% of group			
22 days (66%, 46-82%)(b)	9/38	11/46	4/17	5/28
22.5 days (20.6%, 9-50%)	10/42	5/21	1148	4/22
23 days (13%, 0-25%)	5/21	8/33	8/35	7/39
23.5 days (0%)	0	0	0	1/6*
24 days (0%)	0	0	0	0
24.5 days (0%)	0	0	0	1/6*
Mean gestation length (days)	~22.42	~22.44	~22.59	~(22.72)
Gestation index (%)	100	100	96	71***

a Data were obtained from pages 71 and 85 of the study report. b (mean %, range in %)
* Distribution of gestation length significantly different from controls; $p \leq 0.05$
*** Significantly different from controls; $p<0.001$.

4. Maternal postmortem results:

a. Macroscopic examination: No treatment related effects were observed on macroscopic examinations or on absolute or relative (to body) brain weights.

b. Microscopic examination: No microscopic examinations were performed on the dams.

B. OFFSPRING

1. Viability and clinical signs: Viability data are presented in Table 5a. One female at every dose level was observed to have a total litter loss. No effects of treatment were observed on the mean number of implantations, post-implantation survival index, total mean litter size on PND 1, or sex ratio. Mean live litter size was slightly reduced at 5 mg/kg on PND 1-4 (pre- and post-culling), becoming significant ($p \leq 0.05$) on PND 7-28 (↓9-12%). Live birth, viability, and lactation indices were also slightly reduced (NS). No effects of treatment were observed on viability at 1 or 2.5 mg/kg.

Clinical signs of toxicity (# of litters with offspring showing signs/total # of litters) in pre-weaning pups are presented in Table 5b. At 5 mg/kg, the number of litters in which one or more pups were cold to the touch (6/16), had little or no milk in the stomach (6/16), or were underactive (5/16) were increased compared to controls (0-1/23). A smaller increase in these clinical signs were observed in the 1 and 2.5 mg/kg groups (3/22-23). No other treatment-related clinical signs of toxicity were observed.

Table 5a. F_1 viability[a]

Observation	Dose (mg/kg/day)			
	0	1	2.5	5
Number of litters	23	23	22	16
Total litter loss	1	1	1	1
Mean (±SD) implantations	15.1±1.8	15.3±1.7	16.0±1.8	15.4±1.9
Post-implantation survival index (%)	94.9	95.3	92.5	94.0
Mean (±SD) litter size				
PND 1 (total)	14.6±1.9	14.6±1.7	14.9±2.0	14.6±1.9
PND 1 (live)	14.3±2.0	14.3±1.6	14.7±2.1	13.8±2.3
PND 4[b]	14.2±1.9	13.8±2.0	14.3±2.3	12.4±3.8
PND 4[c]	8.0±0.0	8.0±0.0	8.0±0.0	7.6±1.5
PND 7	8.0±0.2	7.9±0.4	7.8±0.5	7.3±1.5* (19)
PND 11	8.0±0.2	7.9±0.4	7.7±0.8	7.1±1.8* (111)
PND 14	7.9±0.3	7.9±0.4	7.6±1.0	7.1±1.8* (110)
PND 21	7.9±0.3	7.9±0.4	7.6±1.0	7.1±1.8* (110)
PND 28	6.9±0.3	6.9±0.4	6.6±1.0	6.1±1.3** (112)
Mean (±SD) sex ratio (% male)				
PND 1 (total)	49.4±13.3	49.3±15.3	50.2±12.5	49.0±15.3
PND 1 (live)	49.7±13.4	49.4±15.3	50.1±12.6	48.8±15.3
PND 4[b]	49.4±13.7	49.0±15.3	50.1±12.5	48.1±14.9
PND 4[c]	49.5±5.9	49.5±8.0	50.0±0.0	51.1±6.6
PND 21	49.5±7.2	49.5±8.0	50.9±2.9	47.8±14.9
Live birth index (%)	98.5	98.6	98.7	94.7
Viability index (%)	99.2	96.5	97.4	**89.2**
Lactation index (%)				
PND 7	99.5	98.9	97.7	96.9
PND 11	99.5	98.9	96.0	93.8
PND 14	98.9	98.9	95.5	93.8
PND 21	98.9	98.9	95.5	93.8

a Data were obtained from pages 87-89 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.
b Before standardization (culling).
c After standardization (culling).
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

Table 5b. F_1 clinical signs of toxicity (litters with offspring showing signs/total litters) in pre-weaning pups.[a]

Clinical Sign	Dose (mg/kg/day)			
	0	1	2.5	5
Cold to touch	1/23	3/23	3/22	6/16
Little/no milk in stomach	1/23	3/23	3/22	6/16
Underactivity	0/23	3/23	3/22	5/16

a Data were obtained from pages 202-206 of the study report.

2. <u>Body weight</u>: During pre-weaning, body weights were decreased ($p \leq 0.05$) at 5 mg/kg in both sexes on PND 1-21 (↓6-15%; Table 6a). Body weight gains were decreased ($p \leq 0.05$) in the males by 9% on PND 1-17, and in the females by 20% on PND 1-7 and 10% overall (PND 1-21). During post-weaning, body weights were decreased ($p \leq 0.01$) at 5 mg/kg in both sexes on PND 28-65 (↓7-10%; Table 6b), and at 1 and 2.5 mg/kg in both sexes on PND 49-65 (↓4-7%; $p \leq 0.05$). Overall post-weaning (PND 28-65) body weight gains were decreased ($p \leq 0.01$) at ≥1 mg/kg in both sexes (↓6-11%). Food consumption was not reported for the F_1 groups.

Table 6a. Selected mean (±SD) F_1 pre-weaning body weights and body weight gains (g)[a]

Postnatal Day	Dose (mg/kg/day)			
	0	1	2.5	5
Males (n=14-23 litters)				
1	6.7±0.5	6.9±0.7	7.1±0.6	6.1±0.6** (↓9)
4[b]	9.3±1.2	9.4±1.4	9.5±1.5	8.3±1.7* (↓11)
4[c]	9.3±1.3	9.5±1.4	9.6±1.05	8.3±1.7* (↓11)
7	15.2±2.2	15.2±2.7	15.2±3.2	13.1±3.6* (↓14)
14	33.4±3.1	33.1±4.0	32.6±5.5	30.2±4.9* (↓10)
17	41.2±3.7	40.6±4.4	40.1±6.0	37.4±4.8* (↓9)
Days 1-17 gain	34.5±3.5	33.7±3.9	33.1±5.6	31.3±4.4* (↓9)
Overall (Days 1-21) gain	46.4±5.2	45.4±5.8	45.5±8.2	43.4±6.1 (↓6.5%)
Females (n=15-23 litters)				
1	6.2±0.5	6.5±0.7	6.6±0.6	5.8±0.6* (↓6)
4[b]	8.8±1.2	8.9±1.4	8.9±1.5	8.0±1.3
4[c]	8.8±1.2	8.9±1.4	8.9±1.6	8.0±1.3
7	14.4±2.1	14.7±2.7	14.2±3.1	12.4±3.0 (↓14)
11	24.2±2.6	24.2±3.6	23.6±5.0	20.6±4.3** (↓15)
14	32.1±2.9	31.9±4.2	31.2±5.4	27.6±5.1** (↓14)
21	50.8±4.8	50.2±6.0	49.7±8.7	45.6±6.7* (↓10)
Days 1-7 gain	8.2±1.7	8.1±2.1	7.7±2.6 (↓6.1%)	6.6±2.6* (↓20)
Overall (Days 1-21) gain	44.5±4.4	43.7±5.5	43.1±8.3	39.9±6.4* (↓10)

a Data were obtained from pages 90-93 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

b Before standardization (culling).

c After standardization (culling).

* Significantly different from controls; $p<0.05$

** Significantly different from controls; $p<0.01$

Table 6b. Selected mean (±SD) F_1 post-weaning body weights and body weight gains (g)[a]

Postnatal Day	Dose (mg/kg/day)			
	0	1	2.5	5
Males (n=42-58 pups)				
28	90±9	88±11	88±14	84±9** (-7)
42	210±18	201±21	201±28	190±21** (-10)
49	273±27	261±26* (-4)	260±36* (-5)	247±25** (-10)
65	398±31	376±35** (-6)	372±44** (-7)	357±35** (-10)
Overall (Days 28-65) gain	307±26	289±28** (-6)	284±33** (-7)	273±29** (-11)
Females (n=42-57 pups)				
28	81±8	79±10	80±12	75±12** (-7)
42	161±12	156±15	157±20	148±19** (-8)
49	191±13	182±17* (-5)	184±22* (-4)	172±21** (-10)
65	241±16	229±20** (-5)	230±25** (-5)	217±24** (-10)
Overall (Days 28-65) gain	160±15	150±15** (-6)	150±18** (-6)	142±17** (-11)

a Data were obtained from pages 119-120 in the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$

The apparent lower body weights in the low and mid dose groups were not considered to be treatment related by the *ad hoc* DNT review committee. There was no clear dose response between 1 and 2.5 mg/kg/day.

3. Developmental landmarks

a) Sexual maturation: No effects of treatment were observed on either time to preputial separation or time to vaginal patency (Table 7).

Table 7. Mean (±SD) time (days) to sexual maturation in F_1 rats[a]

Parameter	Dose (mg/kg/day)			
	0	1	2.5	5
N (M/F)	58/56	58/57	56/52	42/42
Preputial separation (males)	45.4±2.0	46.6±2.8	46.2±3.5	46.9±3.2
Vaginal opening (females)	34.4±1.8	34.5±2.0	34.2±2.6	34.5±2.6

a Data were obtained from pages 121-122 of the study report.
* Significantly different from controls, $p<0.05$

b) Physical landmarks: Physical landmarks were not reported for the F_1 groups.

4. Behavioral assessments

a) Functional observational battery: On PND 11, the 2.5 mg/kg males and the 5 mg/kg females demonstrated an increased surface righting reflex (1.5 vs 1.1-1.2 controls; Table 8). On PND 21, the 5 mg/kg males displayed a higher level of activity. No other effects of treatment were observed on the arena FOB parameters. No effects of treatment were observed on the handling FOB observations.

Table 8. Functional observational battery results (incidence)[a]

Observation	Dose (mg/kg/day)			
	0	1	2.5	5
Males				
Surface righting reflex (mean score)				
PND 4	2.2	2.0	1.9	2.1
PND 11	1.1	1.0	**1.5**	**1.5**
Activity count (mean score)				
PND 11	4.0	3.3	4.8	3.3
PND 21	4.6	3.3	3.7	**7.5**
PND 35	9.5	11.1	7.5	9.2
PND 45	4.8	6.2	5.5	7.7
PND 60	9.1	9.8	7.3	8.4
Females				
Surface righting reflex (mean score)				
PND 4	2.3	2.4	2.7	2.3
PND 11	1.2	1.2	1.1	**1.5**
Activity count (mean score)				
PND 11	4.0	2.7	5.5	4.2
PND 21	4.5	3.5	4.0	4.5
PND 35	10.5	8.5	7.1	10.1
PND 45	9.6	8.0	5.9	8.8
PND 60	17.5	14.0	12.8	16.3

a Data were obtained from pages 94-99 of the study report. n = 10-13/sex/dose

b) Motor activity: No effects of treatment were observed on motor activity (Tables 9a and b). The higher values observed for PND 17 male controls were attributed to two animals with abnormally elevated (>470) high beam breaks. Several differences ($p \leq 0.05$) in motor activity were noted at various intervals; however, they were considered incidental and unrelated to treatment (Tables 9c, d, e, and f). Habituation was unaffected by treatment.

TABLE 9a. Mean ±SD rearing - high beam breaks per session[a]

Test Day	Dose (mg/kg/day)			
	0	1	2.5	5
Males				
PND 13	1.0±1.7	0.6±1.1	0.3±0.9	0.5±1.2
PND 17	120.5±180.6	52.8±62.1	54.8±51.5	39.1±63.9
PND 22	59.8±43.9	53.8±60.7	54.4±37.9	56.9±43.7
PND 59	313.2±71.2	377.0±165.1	250.9±130.5	307.8±128.2
Females				
PND 13	1.5±2.0	1.0±1.9	0.4±0.9	0.8±2.2
PND 17	48.7±39.4	48.0±71.1	29.6±33.4	52.7±66.7
PND 22	30.0±23.3	57.1±34.4	35.9±15.8	53.1±52.1
PND 59	324.9±112.9	316.5±83.7	335.9±111.2	332.8±119.5

a Data were obtained from pages 103-110 and 301 of the study report. n = 9-12

TABLE 9b. Mean ±SD Total cage floor - low beam breaks per session[a]

Test Day	Dose (mg/kg/day)			
	0	1	2.5	5
Males				
PND 13	285.3±247.2	209.4±244.3	156.1±113.8	238.5±197.3
PND 17	552.6±279.4	444.8±325.4	497.4±417.8	332.0±312.8
PND 22	258.6±151.5	219.9±150.3	324.2±271.0	244.3±164.1
PND 59	1052.3±227.7	1182.8±477.5	893.4±356.8	984.2±307.2
Females				
PND 13	205.6±149.6	182.4±128.0	167.7±131.2	235.2±210.9
PND 17	552.1±351.9	352.7±260.0	251.5±119.8	557.2±347.0
PND 22	192.7±132.9	301.5±172.4	243.7±116.3	284.9±199.4
PND 59	1452.6±529.6	1405.9±318.1	1375.7±338.5	1312.7±264.8

a Data were obtained from pages 103-110 of the study report. n = 9-12

Table 9c. Mean (±SD) sub-session data in males (rearing - high beam breaks per session)[a]

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 13	1	0.3±0.6	0.1±0.3	0.3±0.9	0.4±1.2
	2	0.4±0.9	0.3±0.9	0.0±0.0	0.0±0.0
	3	0.1±0.3	0.0±0.0	0.0±0.0	0.1±0.3
	4	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0
	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	6	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0
	7	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0
	8	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3
	9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	10	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PND 17	1	10.5±11.0	7.8±11.8	8.3±11.6	8.0±12.6
	2	9.2±10.8	6.0±8.1	3.0±4.3	4.2±7.8
	3	8.3±14.9	5.8±7.6	6.8±7.9	5.3±9.0
	4	17.5±29.1	9.5±12.7	7.5±11.7	8.2±13.3
	5	9.4±17.4	3.0±5.8	5.2±6.7	3.7±5.9
	6	13.5±24.1	2.6±4.7	7.8±16.8	3.2±6.5
	7	9.2±15.7	5.7±11.5	2.3±3.5	0.5±1.7* (195)
	8	15.2±29.1	3.8±7.1	7.1±11.8	1.5±3.9
	9	17.0±33.3	2.1±4.3	2.9±6.1	1.3±3.8
	10	10.8±16.6	6.4±12.5	4.1±9.2	3.3±10.9
PND 22	1	17.0±14.4	13.1±12.0	9.5±11.7	12.3±8.3
	2	11.2±9.6	13.7±11.9	9.7±8.0	11.7±11.5
	3	9.8±8.3	7.7±14.8	9.0±7.1	5.8±6.0
	4	5.8±5.4	4.7±10.4	6.5±7.7	5.5±5.5
	5	2.7±5.2	3.8±7.3	4.8±5.8	4.1±7.9
	6	1.9±4.1	3.0±6.9	5.4±8.8	4.6±7.4
	7	3.2±5.6	3.9±7.5	2.0±4.6	3.4±7.2
	8	3.9±6.3	1.7±3.3	0.7±1.7	2.6±3.8
	9	1.4±3.0	0.9±1.5	4.0±7.6	1.9±4.6
	10	3.0±6.0	1.4±3.8	2.8±5.9	5.0±9.5

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 59	1	58.4±26.9	66.8±23.1	46.5±21.8	48.3±24.7
	2	54.5±11.5	66.7±30.0	53.3±26.9	56.3±25.3
	3	49.3±15.2	48.6±30.9	41.1±19.2	48.3±22.3
	4	43.1±21.1	41.0±23.1	31.3±24.7	34.1±25.4
	5	28.5±11.8	36.3±20.4	23.4±17.7	30.2±17.4
	6	21.4±17.6	29.4±23.2	18.8±18.8	25.1±22.8
	7	21.3±17.0	26.0±21.6	13.3±21.4	21.4±12.6
	8	15.5±12.8	22.8±23.1	6.3±9.3	20.0±16.5
	9	12.0±15.4	22.5±21.8	9.7±13.1	7.8±9.6
	10	9.2±12.6	17.0±15.9	7.3±10.4	16.6±18.2

a Data were obtained from pages 103-110 of the study report. n = 9-12

* Significantly different from controls; $p \leq 0.05$

Table 9d. Mean (±SD) sub-session data in females (rearing - high beam breaks per session)[a]

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 13	1	0.8±1.7	0.4±0.7	0.2±0.7	0.4±1.3
	2	0.1±0.3	0.0±0.0	0.2±0.7	0.0±0.0
	3	0.0±0.0	0.3±0.9	0.0±0.0	0.0±0.0
	4	0.1±0.3	0.0±0.0	0.0±0.0	0.2±0.6
	5	0.4±0.7	0.0±0.0	0.0±0.0	0.0±0.0
	6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	7	0.0±0.0	0.3±0.9	0.0±0.0	0.1±0.3
	8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	9	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0
	10	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3
PND 17	1	5.0±5.7	3.6±5.6	9.1±15.8	6.7±7.5
	2	6.4±6.5	8.8±9.8	4.8±9.8	6.6±12.5
	3	8.1±10.9	8.7±14.8	4.5±7.9	8.6±12.9
	4	6.8±5.8	2.3±4.0	3.1±5.1	4.8±5.7
	5	3.7±4.0	7.5±10.5	2.8±6.2	6.0±9.2
	6	3.6±5.4	5.5±11.1	0.7±2.2	2.3±3.9
	7	4.9±5.6	3.0±7.1	0.8±1.3	5.1±10.0
	8	4.6±6.4	0.5±1.8	1.4±4.4	3.6±5.8
	9	3.6±5.4	5.7±12.8	0.2±0.6	2.5±4.1
	10	1.9±5.0	2.3±5.7	2.2±7.0	6.5±13.4

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 22	1	8.0±4.5	11.2±8.0	7.5±8.6	6.1±11.9
	2	7.3±5.3	9.4±7.3	7.0±6.1	9.0±10.3
	3	4.3±3.9	5.1±5.6	4.1±4.1	5.2±9.5
	4	3.1±8.1	6.7±7.8	2.8±4.5	5.4±5.9
	5	2.4±2.9	4.8±6.5	4.2±3.7	5.3±7.0
	6	1.4±2.4	5.1±7.9	2.7±2.8	6.2±10.6
	7	1.4±3.2	9.6±10.6	1.2±1.7	5.8±8.5
	8	0.2±0.6	2.3±3.4	3.7±8.8	6.4±9.7* (13100)
	9	0.4±1.2	1.7±2.6	2.2±3.5	2.2±4.1
	10	1.7±4.6	1.2±3.3	0.5±1.3	1.5±3.4
PND 59	1	63.0±20.0	64.1±21.3	48.6±21.6	52.7±16.1
	2	47.7±21.1	43.2±16.1	55.3±17.5	52.3±15.4
	3	47.5±18.2	44.7±11.7	50.9±22.5	40.3±28.0
	4	30.2±13.7	41.7±26.1	36.3±15.3	36.4±19.0
	5	33.8±18.2	33.3±18.3	28.0±13.8	31.9±12.2
	6	24.3±10.1	22.5±10.8	29.3±16.5	32.3±16.3
	7	24.4±18.2	20.1±11.7	29.2±18.0	24.1±19.9
	8	20.5±20.8	23.1±22.3	20.6±17.8	26.9±19.5
	9	18.8±18.3	12.2±10.2	22.3±22.7	21.4±17.8
	10	14.6±14.0	11.6±12.4	15.4±16.2	14.5±10.6

a Data were obtained from pages 103-110 of the study report. n = 9-12

* Significantly different from controls; $p \leq 0.05$

Table 9e. Mean (±SD) sub-session data in males (cage floor - low beam breaks per session)[a]

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PD 13	1	50.1±30.9	70.5±40.7	69.7±70.4	63.6±47.4
	2	31.0±39.6	18.6±24.3	18.1±31.7	51.8±54.7
	3	43.5±52.2	15.2±21.0	18.4±35.3	28.2±43.5
	4	23.8±27.6	14.7±23.1	8.3±12.1	16.5±30.1
	5	41.8±70.7	18.1±44.7	12.2±16.1	13.9±21.8
	6	15.0±22.9	22.7±60.7	11.6±21.7	14.7±18.7
	7	32.5±32.6	8.3±13.5 (174)	1.3±1.9* (196)	4.5±5.4* (186)
	8	10.8±33.3	3..2±5.2	5.2±8.4	20.2±35.8
	9	5.0±9.3	12.1±28.4	3.5±6.3	17.1±38.5
	10	31.7±52.8	26.0±63.3	7.8±13.9	8.0±11.2

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 17	1	96.6±50.7	98.3±37.3	72.8±48.7	90.8±50.2
	2	69.6±42.7	64.9±18.4	53.1±37.6	45.6±42.4
	3	53.5±37.7	70.5±81.5	78.4±62.3	56.3±52.7
	4	75.5±64.0	47.5±59.2	60.5±72.8	37.6±43.8
	5	34.5±32.4	28.8±50.1	44.5±60.2	27.0±40.6
	6	52.7±59.6	35.2±52.7	39.3±57.4	19.2±34.1
	7	37.6±39.2	32.8±42.7	30.2±55.1	12.3±29.8
	8	50.4±43.3	23.7±38.1	46.1±54.0	25.5±58.6
	9	36.1±46.6	17.5±34.2	35.5±48.4	11.0±25.7
	10	46.2±72.3	25.7±44.8	37.2±55.0	6.8±16.4
PND 22	1	76.6±35.6	68.8±30.3	49.8±34.1	63.4±37.8
	2	37.0±22.1	42.9±15.8	47.6±36.9	45.3±42.9
	3	31.7±23.0	26.3±27.6	48.2±36.7	17.3±14.2
	4	23.3±16.8	20.8±24.3	49.8±49.0	24.3±27.0
	5	24.8±29.0	17.8±25.8	38.8±47.7	15.6±18.2
	6	13.6±20.9	8.5±14.7	27.5±37.2	17.0±20.5
	7	18.4±22.4	16.5±26.7	17.2±32.6	16.8±32.8
	8	14.9±21.7	7.3±13.1	7.8±16.1	18.3±25.3
	9	5.9±13.7	5.4±11.7	18.7±38.2	9.9±17.9
	10	12.4±27.4	5.7±13.0	19.0±31.5	16.3±25.0
PND 59	1	167.9±50.9	190.3±47.1	134.8±53.3	149.3±47.1
	2	162.0±37.9	186.7±55.7	147.9±49.4	151.9±56.0
	3	136.2±18.8	151.8±60.4	118.1±30.5	147.4±44.2
	4	121.3±39.2	129.6±50.8	121.3±43.5	114.2±48.5
	5	113.3±37.5	123.7±66.9	110.0±52.6	110.9±52.7
	6	101.2±40.6	100.4±92.1	75.9±60.7	80.3±47.1
	7	84.0±45.2	69.3±54.6	58.1±56.7	84.8±52.3
	8	60.3±46.1	88.7±63.7	44.4±47.5	57.3±41.5
	9	58.9±62.3	82.4±72.3	43.8±48.8	38.6±39.6
	10	47.3±33.3	59.9±49.7	39.2±47.3	49.5±47.2

a Data were obtained from pages 103-110 of the study report. n = 9-12

* Significantly different from controls; $p \leq 0.05$

Table 9f. Mean (±SD) sub-session data in females (cage floor - low beam breaks per session)[a]

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 13	1	61.5±31.5	60.6±51.6	56.7±44.0	66.8±37.6
	2	22.8±27.4	31.0±37.7	35.2±43.6	29.3±49.4
	3	17.9±25.9	23.8±27.5	22.1±26.8	25.1±53.9
	4	22.9±23.9	13.7±16.5	12.7±22.5	22.5±28.8
	5	22.3±28.7	14.8±21.1	19.7±45.7	19.4±21.8
	6	23.8±43.7	15.2±29.3	3.7±8.9	10.0±14.4
	7	15.0±33.8	13.3±23.9	5.6±14.1	15.4±26.0
	8	2.1±5.3	2.1±3.8	6.6±13.1	31.3±46.4
	9	9.3±22.5	1.6±2.3	0.0±0.0	4.8±11.1
	10	8.0±23.6	6.3±9.3	5.6±11.2	10.6±14.9
PND 17	1	90.6±53.4	80.8±48.5	66.2±45.2	81.6±42.2
	2	79.1±39.3	76.5±60.1	37.3±49.1	68.3±47.3
	3	69.2±61.6	67.6±71.9	28.2±29.4	67.1±52.9
	4	47.5±37.4	31.4±32.0	49.5±47.3	53.0±54.0
	5	36.2±33.3	30.4±33.1	10.9±13.4	73.8±61.5
	6	48.9±50.9	12.9±21.7	14.5±31.6	41.8±45.6
	7	49.1±54.8	16.4±31.7	28.0±47.9	44.2±69.5
	8	52.4±60.5	6.4±14.3	2.5±5.4	43.8±53.0
	9	41.7±54.5	21.1±44.3	2.1±4.2	25.3±52.2
	10	37.4±47.5	9.3±23.7	12.3±38.9	58.3±73.2
PND 22	1	62.2±32.4	64.2±27.9	57.2±37.0	47.1±34.7
	2	37.0±22.2	36.7±20.7	34.4±24.2	42.9±37.9
	3	25.8±23.7	25.1±18.5	28.5±20.0	25.0±18.0
	4	14.5±21.6	44.1±36.9	15.1±18.5	26.6±24.8
	5	16.0±21.0	29.0±30.9	24.2±15.5	28.9±28.4
	6	13.6±24.0	25.3±23.9	27.5±24.1	25.1±30.4
	7	7.5±16.6	32.1±27.9	15.0±15.0	24.5±27.1
	8	3.0±9.9	20.3±29.0	17.3±29.1	30.2±34.7* (1907)
	9	2.9±7.0	14.5±17.4	16.7±24.3	19.3±24.1
	10	10.2±22.6	10.3±16.7	7.8±13.2	15.3±33.4

Sub-session		Dose (mg/kg/day)			
		Control	1	2.5	5
PND 59	1	220.5±31.9	227.6±32.0	168.1±37.4** (↓167)	179.7±38.7** (↓185)
	2	186.8±57.1	188.3±49.1	203.0±50.0	175.9±27.7
	3	175.7±65.3	174.9±61.3	191.7±47.1	152.1±34.0
	4	164.1±57.5	163.8±55.0	153.3±44.5	143.4±42.8
	5	150.9±85.3	143.6±66.0	126.5±48.1	147.5±29.9
	6	122.0±77.7	118.0±35.0	126.7±49.6	132.7±41.3
	7	127.1±64.3	112.4±45.0	128.0±57.4	106.3±62.0
	8	106.5±90.5	92.5±45.7	95.4±51.3	102.4±42.1
	9	115.6±64.3	91.2±41.9	99.7±62.9	88.2±64.2
	10	83.3±54.9	93.6±52.7	83.3±84.7	84.5±44.5

a Data were obtained from pages 103-110 of the study report. n = 9-12
Significantly different from controls; $p \leq 0.05$

c) Auditory startle reflex

A. Habituation: On PND 23/24, no effect of treatment was observed on auditory startle reflex habituation (Table 10a). However, on PND 60/61, an apparent effect of treatment was noted in the 5 mg/kg/day group. Males demonstrated an *increased* mean startle amplitude for each block of 10 trials (11-34%, not significant [NS] for Blocks 1-4; 63%, $p \leq 0.05$ for Block 5). Females displayed a *decreased* mean startle amplitude for each block of trials (18 to 34%, NS for Blocks 1-3 and 5; 37%, $p \leq 0.05$ for Block 4). The study author also asserts (see page 46 of the study report) that "a clear difference in performance between the groups was apparent for both sexes: among males, 9/11 Controls showed > 20% overall reduction in the mean amplitude of the startle response with just one animal showing no reduction at all compared with 5/11 and 4 animals respectively in the 5.0 mg/kg/day group. Among females, 8/12 Controls showed > 10% reduction in the mean startle amplitude with just two animals showing no reduction at all compared with 4/10 and 4 animals respectively in the 5.0 mg/kg/day dose group".

Thus, the males and females are responding in the opposite direction with regard to the auditory startle reflex. The males in the high dose group were higher than the historical control range values based on two studies. The study control group was also higher in some blocks than the historical control range. Among females, the high dose group appeared to be near the historical controls whereas the study control group appeared to be higher than the historical control range. The higher values in the historical control range may have contributed to the appearance of lower values for the high dose group females.

Table 10a. Mean (±SD) auditory startle reflex peak amplitude data (g)[a]

Block		Dose (mg/kg/day)				
		0	1	2.5	5	Historical
Males						
PND 23/24	1	237.7±112.0	213.8±101.8	244.2±112.5	221.8±91.1	
	2	211.9±107.3	168.9±64.3	218.8±102.8	182.6±85.9	
	3	181.2±79.7	146.6±52.7	203.6±101.9	181.2±65.4	
	4	185.2±80.5	137.6±46.6	172.5±63.3	156.3±65.1	
	5	181.1±74.7	129.9±43.1	168.9±58.1	147.0±46.4	
PND 60/61	1	48.5±20.5	48.3±21.1	49.6±22.6	53.7±35.0 (↑11%)	42.2-48.1
	2	38.6±16.6	41.3±19.5	31.1±9.0	49.6±22.1 (↑28%)	35.0-35.8
	3	37.9±11.1	42.4±24.5	38.1±12.6	50.6±37.2 (↑34%)	31.9-36.9
	4	38.6±7.8	36.1±17.7	39.1±14.9	55.1±39.8 (↑43%)	27.9-32.3
	5	30.7±5.9	38.1±19.0	42.2±19.5	50.0±31.3* (↑63)	34.2-34.7
Females						
PND 23/24	1	228.9±98.3	181.4±68.5	263.6±61.2	173.7±52.5	
	2	177.4±69.3	161.4±76.4	220.7±84.1	152.5±32.6	
	3	173.3±67.4	145.1±60.5	232.0±81.2	142.9±42.2	
	4	199.9±58.2	147.3±60.0	222.4±78.3	154.3±41.6	
	5	183.3±63.5	151.0±57.6	207.4±65.2	137.3±39.0	
PND 60/61	1	47.9±24.4	41.6±15.3	41.6±17.0	31.7±9.7 (↓34%)	36.6-45.6
	2	42.5±20.2	38.3±15.2	37.7±18.6	32.1±9.2 (↓24%)	33.2-36.4
	3	38.0±18.8	36.6±15.2	32.4±15.6	31.1±14.0 (↓18%)	31.-35.5
	4	44.6±16.9	36.9±17.4	37.7±18.2	27.9±12.1* (↓37)	29.0-30.1
	5	36.1±23.2	31.7±12.1	40.2±16.0	28.3±7.5 (↓22%)	29.6-29.7

a Data obtained from pages 115-116 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses. n = 10-12

* Significantly different from control; $p<0.05$

The historical control data were provided in the report on the same tables as the data for this study.

B. Pre-pulse inhibition data.

On PND 23/24, no effect of treatment was observed on auditory startle reflex pre-pulse inhibition (Table 10b). However, on PND 60/61, an apparent effect of treatment was noted in the 5 mg/kg group. Males and females demonstrated an *increased* pre-pulse inhibition. (not significant [NS] for males; $p \leq 0.01$ for females). The study author also asserted that "There was a clear difference in performance of the groups: among males, 7/8 animals in the 5.0 mg/kg/day dose group showed > 45% inhibition compared with just 4/12 Controls; among females, 7/7 animals in the 5.0 mg/kg/day dose group showed > 40% inhibition compared with just 3/11 Controls." The study author also asserted that "the mean amplitude for the stimulus

without pre-pulse for males and females in the 5.0 mg/kg/day group were noticeably higher than in the Background Control range with the values for females being significantly higher than in the concurrent Controls. It was noted that the mean amplitude for the stimulus with pre-pulse was similar in the Control and 5.0 mg/kg/day group for both sexes."

TABLE 10b. Mean (±SD) auditory startle reflex pre-pulse inhibition data (g)[a]

Block		Dose (mg/kg/day)				
		0	1	2.5	5	Historical
Males						
PND 23/24	Without pre-pulse	346.3±55.5	353.1±105.9	388.2±145.4	279.8±61.4	
	With pre-pulse	248.8±50.3	250.4±77.0	287.4±84.4	189.3±58.7	
	% Inhibition	27.8±11	28.8±7.7	23.5±10.9	32.1±15.1	
PND 60/61	Without pre-pulse	74.7±44.8	80.5±49.7	78.6±37.1	89.4±39.5	69.9-71.2
	With pre-pulse	47.2±33.9	52.6±35	54.1±24.1	43.5±17.4	38.4-48.4
	% Inhibition	36.6±19.1	30.3±30.0	28.6±20.4	49.9±8.9	33.3-42.5
Females						
PND 23/24	Without pre-pulse	308.6±92.1	260.3±76.6	337.1±94.6	367.0±164.7	
	With pre-pulse	232.1±67.7	193.7±61.5	243.7±80.2	266.1±133.1	
	% Inhibition	24.4±7.9	24.9±13.9	26.9±17.8	26.8±15.1	
PND 60/61	Without pre-pulse	63.2±17.5	72.4±23.9	79.3±27.8	**99.9±32.9**** (↑58)	53..-60.0
	With pre-pulse	46.4±14.0	43.8±15.9	45.9±18.1	50.1±18.6	30.8-42.9
	% Inhibition	25.2±16.1	38.1±15.9	39.3±20.7	**49.3±8.9**** (↑96)	27.9-40.4

a Data were obtained from pages 117-118 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses. n = 7-12

** Significantly different from control; p<0.01

d) <u>Learning and memory testing</u>: No treatment-related effects were observed on learning and memory as determined by testing in the Morris water maze (Tables 11a and b). The control data indicate that the male and female rats learned the tasks.

Table 11a. Morris water maze performance - males (mean ± SD)[a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	1	2.5	5
PND 23-24					
Test day 1	Trial time (sec)	71.4±18.2	58.4±21.3	76.2±15.6	73.9±15.3
	No. failed trials	1.8±1	1.4±1	2.1±1	2.0±0.9
	No. sector entries	17.3±3.1	16.4±4.3	18.3±3.3	17.7±4.3
	% of animals with at least 1 failed trial	90.9	81.8	90.0	100
Test day 2	Trial time (sec)	47.1±16.7	49.4±20.7	36.1±24.4	35.7±10.3
	No. failed trials	1±0.8	0.9±0.8	0.6±0.7	0.1±0.3**
	No. sector entries	12.5±2.7	12.8±4.2	11±6.3	11.7±3.4
	% of animals with at least 1 failed trial	72.7	63.6	50.0	10.0
Test day 3	Trial time (sec)	27.1±17.3	29.8±12.7	40.2±29.4	33.0±17.2
	No. failed trials	0.3±0.5	0.2±0.4	0.6±1.1	0.3±0.5
	No. sector entries	8.1±3.5	9.8±3.7	11.5±6.6	11.4±5.3
	% of animals with at least 1 failed trial	27.3	18.2	30.0	30.0
Test day 4	Trial time (sec)	18.0±10.6	16.4±6.6	34.9±24.3	15.8±6.3
	No. failed trials	0.0±0.0	0.0±0.0	0.4±0.7	0.0±0.0
	No. sector entries	6.4±2.4	6.1±1.9	11.2±5.7* (↑75)	6.3±2.4
	% of animals with at least 1 failed trial	0.0	0.0	30.0	0.0
PND 60/61					
Test day 1	Trial time (sec)	68.6±15.7	63.6±14.9	71±11.9	69.9±19.8
	No. failed trials	1.5±0.9	1.3±1	1.8±0.8	1.8±0.9
	No. sector entries	14.5±3.7	13.5±3.3	15.4±1.9	14.2±3.4
	% of animals with at least 1 failed trial	91.7	75	100	90.0
Test day 2	Trial time (sec)	44.2±25.6	35.5±19.7	45±26.9	40.4±18.5
	No. failed trials	0.7±1.1	0.5±0.7	0.9±1.2	0.4±0.7
	No. sector entries	10.6±4.2	9.2±3.3	11.4±6.4	10.0±3.6
	% of animals with at least 1 failed trial	33.3	41.7	50.0	30.0
Test day 3	Trial time (sec)	33.1±23.3	27.0±9.2	31.5±17.5	33.1±21.4
	No. failed trials	0.3±0.9	0.1±0.3	0.3±0.7	0.4±0.7
	No. sector entries	8.3±4.0	7.3±2.7	8.6±3.7	8.6±4.1
	% of animals with at least 1 failed trial	16.7	8.3	25	30.0
Test day 4	Trial time (sec)	23.0±19.0	18.4±12.2	20.6±14.2	22.8±12.0
	No. failed trials	0.3±0.7	0.1±0.3	0.1±0.3	0.0±0.0
	No. sector entries	6.0±3.2	4.8±2.3	5.6±2.4	6.0±3.0
	% of animals with at least 1 failed trial	25	8.3	8.3	0.0

a Data were obtained from pages 111 and 113 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses. n = 10-12

* Significantly different from control; $p<0.05$

** Significantly different from control; $p<0.01$

Table 11b. Morris water maze performance - females (mean ± SD)[a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	1	2.5	5
PND 23-24					
Test day 1	Trial time (sec)	65.6±20.5	52.9±15.4	61.6±15.5	63.0±20.1
	No. failed trials	1.6±1.1	1.1±0.8	1.3±0.9	1.4±1.2
	No. sector entries	16.1±5.4	15.2±3.5	16.3±4.1	16.5±4.2
	% of animals with at least 1 failed trial	83.3	83.3	83.3	72.7
Test day 2	Trial time (sec)	40.8±19.3	48.2±21.6	52.2±22.9	40.9±22.3
	No. failed trials	0.6±0.9	0.8±1	0.8±1.1	0.6±0.8
	No. sector entries	12.3±4.4	14.1±5	16.3±5.5	12.3±5.6
	% of animals with at least 1 failed trial	33.3	41.7	41.7	45.5
Test day 3	Trial time (sec)	22.2±9.9	23.5±16.3	28.7±17.2	33.8±20.1
	No. failed trials	0.0±0.0	0.2±0.4	0.3±0.5	0.4±0.7
	No. sector entries	7.1±2.8	7.7±4.6	8.9±4.1	11.3±6.0
	% of animals with at least 1 failed trial	0.0	16.7	25	27.3
Test day 4	Trial time (sec)	23.6±21.5	18.0±6.7	19.9±14.0	25.5±17.1
	No. failed trials	0.2±0.4	0.1±0.3	0.0±0.0	0.2±0.6
	No. sector entries	7.8±5.6	6.1±1.4	7.8±4.5	8.4±4.3
	% of animals with at least 1 failed trial	16.7	8.3	0.0	9.1
PND 60/61					
Test day 1	Trial time (sec)	73.6±19.1	69.4±19.1	84.4±9.5	74.2±15.6
	No. failed trials	1.9±0.9	1.6±1.1	2.7±0.5	2.1±0.9
	No. sector entries	15±3.6	14.9±4.8	19.9±3.1* (↑33)	18.7±4.8* (↑25)
	% of animals with at least 1 failed trial	100	81.8	100.0	100.0
Test day 2	Trial time (sec)	40.9±16.7	52.9±20.8	34.1±19.1	33.9±14.9
	No. failed trials	0.5±0.7	0.7±1.1	0.1±0.3	0.2±0.4
	No. sector entries	11.4±5.3	13.8±4.4	9.1±4.5	9.8±3.8
	% of animals with at least 1 failed trial	40.0	36.4	10.0	20.0
Test day 3	Trial time (sec)	29.8±11.8	33.0±21.6	34.7±11.8	30.0±20.2
	No. failed trials	0.2±0.4	0.5±0.7	0.2±0.4	0.1±0.3
	No. sector entries	8.4±2.7	7.8±3.5	9.2±3.1	8.3±4.7
	% of animals with at least 1 failed trial	20.0	36.4	20.0	10.0
Test day 4	Trial time (sec)	24.4±11.4	30.7±13.2	25.6±10.2	19.7±9.3
	No. failed trials	0.0±0.0	0.3±0.5	0.2±0.4	0.0±0.0
	No. sector entries	7.0±3.1	7.7±2.6	8.0±3.2	5.9±2.3
	% of animals with at least 1 failed trial	0.0	27.3	20.0	0.0

a Data were obtained from pages 112 and 114 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses. n = 10-12

* Significantly different from control; $p<0.05$.

5. Postmortem results

a) **Brain weights:** No effects of treatment were observed on brain weight in the F_1 animals (Table 12).

Table 12. Mean (±SD) absolute and relative (to body) brain weights in F_1 rats[a]

Parameter	Dose (mg/kg/day)			
	0	1	2.5	5
Males				
PND 21 (n=10-11)				
Terminal body weight (g)	51.2±5.0	50.6±6.8	49.4±10.1	49.8±9.5
Absolute brain weight (g)	1.57±0.1	1.53±0.1	1.56±0.1	1.53±0.1
Relative (to body) brain weight (%)	3.09±0.4	3.05±0.3	3.31±0.8	3.21±0.8
Brain length (mm)	19.1±1.3	18.8±0.9	18.7±1.4	18.4±0.6
Brain width (mm)	14.8±0.4	14.8±0.4	14.8±0.4	14.8±0.5
PND 65 (n=32-48)				
Terminal body weight (g)	391.5±29.3	370.8±36.1	368.7±40.8	368.7±32.0
Brain weight (g)	2.12±0.1	2.11±0.1	2.09±0.1	2.09±0.1
Brain-to-body weight ratio	0.54±0.0	0.57±0.0	0.57±0.0	0.57±0.0
PND 65 (post-perfusion; n=10)				
Terminal body weight (g)	403.5±34.6	379.1±23.4	375.3±56.6	359.9±43.7
Brain weight (g)	2.00±0.09	1.98±0.09	1.91±0.12	1.92±0.14
Brain-to-body weight ratio	0.50±0.04	0.52±0.03	0.52±0.09	0.54±0.05
Brain length (mm)	21±0.6	21±0.4	20.9±0.6	20.7±0.4
Brain width (mm)	15.2±0.4	15.4±0.4	15.6±0.7	15.4±0.5
Females				
PND 21 (n=10-13)				
Terminal body weight (g)	53.2±8.6	51±5.7	50.6±8.8	45.1±7.7
Brain weight (g)	1.49±0.1	1.52±0.1	1.47±0.1	1.45±0.1
Brain-to-body weight ratio	2.86±0.4	3.00±0.3	2.97±0.5	3.28±0.5
Brain length (mm)	18.1±0.3	18.3±0.5	18.4±1	18.3±1.2
Brain length (mm)	14.7±0.4	15.0±0.5	14.7±0.9	14.6±0.4
PND 65 (n=32-47)				
Terminal body weight (g)	237.1±14.8	226.8±21.4	231±23.8	218.6±24.4
Brain weight (g)	1.95±0.1	1.95±0.1	1.94±0.1	1.88±0.1
Brain-to-body weight ratio	0.82±0.1	0.87±0.1	0.85±0.1	0.87±0.1

Parameter	Dose (mg/kg/day)			
	0	1	2.5	5
PND 65 (post-perfusion; n=10)				
Terminal body weight (g)	244.2±21.8	230.1±9.5	226.8±28.0	209.0±22.7
Brain weight (g)	1.88±0.08	1.82±0.10	1.87±0.15	1.79±0.10
Brain-to-body weight ratio	0.78±0.08	0.79±0.04	0.83±0.12	0.86±0.10
Brain length (mm)	20.5±0.4	20.6±0.5	20.2±0.7	20.0±1
Brain width (mm)	15.1±0.3	15.0±0.4	15.1±0.6	14.9±0.4

a Data were obtained from pages 123-124 and 126-128 of the study report.

b) Neuropathology

1) Macroscopic examination: No treatment-related gross pathological findings were noted in the F_1 animals.

2) Microscopic examination: No effects of treatment were observed on either brain morphometry (Table 13) or microscopic pathology in the F_1 animals.

Table 13. Mean (±SD) morphometric measurements (mm) in F_1 animals[a]

Parameter	Dose (mg/kg/day)			
	Males		Females	
	0	5	0	5
Day 21				
Neocortex	1.92±0.11	1.90±0.14	1.90±0.09	1.83±0.10
Hippocampus	1.80±0.17	1.74±0.10	1.69±0.16	1.69±0.18
Corpus callosum	0.23±0.05	0.25±0.09	0.20±0.05	0.23±0.05
Cerebellum	0.84±0.13	0.91±0.07	0.88±0.08	0.82±0.06
Day 65				
Neocortex	1.88±0.20	1.80±0.19	1.80±0.15	1.72±0.14
Hippocampus	2.00±0.19	1.92±0.20	1.76±0.12	1.80±0.14
Corpus callosum	0.27±0.07	0.31±0.08	0.23±0.02	0.25±0.05
Cerebellum	0.89±0.10	0.88±0.07	0.92±0.09	0.86±0.07

a Data were obtained from pages 125 and 129 of the study report. n = 9-10

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that treatment with 5 mg/kg/day TPTH was not associated with any selective developmental neurotoxicity. There appeared to be a slight delay in the development of the surface righting reflex on PND 11, and a clustering of differences in performance on tests of the auditory startle response at PND 60/61 which were considered suggestive of an influence of maternal treatment. These findings occurred at a dosage associated with the sacrifice of 6 dams due to poor condition around the time of birth or parturition difficulties, slightly impaired offspring survival, and body weight performance and signs of poor general condition among litters in some offspring. According to the study author (page 14 of the study report, 5 mg/kg/day TPTH was considered to be the clear NOAEL for the morphological development of the nervous system in the CD rat following maternal treatment by oral gavage from GD 6 to LD 20. A dose of 2.5 mg/kg/day TPTH was considered to be the clear NOAEL for functional development of the nervous system.

B. REVIEWER COMMENTS:

1. **Maternal toxicity.** The clinical signs of the single dam in the 2.5 mg/kg/day dose group were the same as seen in the several dams at the next higher dose. Based on the results of several developmental toxicity studies with TPTH, such signs might be expected from repeated gavage with TPTH during gestation. Therefore, HED reviewers conclude that the single dam that required sacrifice in the 2.5 mg/kg/day had reactions related to treatment and thus the NOAEL for the maternal aspects of this study is 1 mg/kg/day and the LOAEL is 2.5 mg/kg/day. This conclusion differs from that of the study author and contractor reviewer.

2. **Adequacy of dosing.** Since the dams showed definite reactions to treatment, dosing to the dams was adequate and approaching excessive in the high dose group.

The preliminary milk secretion study (2003, MRID # 45890101) demonstrated that only about 0.09 μg/mL of TPTH was present in the dams milk and available for dosing the pups. This is a small amount and less than the stated doses to the dams. Thus, the pups received only a small amount of TPTH during lactation. If TPTH's optimal window for causing adverse developmental effects was during lactation, than this window may have been missed since too little TPTH was transferred to the milk for proper assessment.

3. **Developmental toxicity.**

Starting at about day 49 and persisting to termination, the low (4.4 to 5.5% for males, and 4.7 to 5% for females, $p < 0.05$ of 0.01) and mid (4.8 to 6.5% for males and 3.7% to 4.6% for females, $p < 0.05$ or 0.01) dose group body weights were decreased. Mean body weight gain over the period of days 28 to 65 were also decreased for the low (6%, both sexes, $p < 0.01$) and mid (6% females and 7% males, both $p < 0.01$) dose groups. However, the HED reviewers determined that there was a lack of a clear dose response to justify concluding that there were treatment related body weight effects at the 1 and 2.5 mg/kg/day dose groups.

The mean litter size was similar at birth for all groups but mean pup weight at birth was slightly lower (↓9% for males and ↓6% for females, both $p < 0.05$) in the high dose group. During lactation, the high dose group pups reached 14 to 15% lower body weights and weight gain during lactation was

reduced (15% for males and 20% for females for days 1 to 7). The high dose group maintained a 7 to 10% differences in post weaning body weight until day 65 accompanied with a decrease of 11% in body gain over this period. Treatment had no adverse effects on survival, clinical signs, food consumption, developmental landmarks, motor activity, auditory startle response, learning and memory, brain weights, brain morphology or neuropathology at any dose.

The maternal LOAEL is 2.5 mg/kg/day, based on clinical condition (requiring sacrifice) in one animal with conditions similar to dams at the next higher dose. The maternal NOAEL is 1 mg/kg/day.

The offspring LOAEL is 5 mg/kg/day based on decreases in body weight and body weight gain. The offspring NOAEL is 2.5 mg/kg/day.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted but do not affect the conclusions of this review:

- Homogeneity and stability data were not provided. Since the dose was in corn oil and there were effects noted in the high dose as expected for TPTH, this should not compromise the interpretation of the study.

13544

R117687

Chemical: **Fentin hydroxide**

PC Code:
083601
HED File Code: **13000 Tox Reviews**
Memo Date: **10/3/2005**
File ID:
Accession #: **412-06-0009**

HED Records Reference Center
2/17/2006