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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

TXR# 0050608

DATE: September 26, 2005

MEMORANDUM

SUBJECT: **METHYL PARATHION** - Review of Developmental Neurotoxicity Study in Rats (MRID 45630301)

PC Code: 053501
DP Barcode #: D281892

From: Robert J. Mitkus
Registration Action Branch I
Health Effects Division (7509C)

Thru: P.V. Shah, Branch Senior Scientist
Registration Action Branch I
Health Effects Division (7509C)

To: Susan Lewis
Reregistration Branch I
Special Review and Reregistration Division (7508C)

ACTION REQUESTED: The Special Review and Reregistration Division (SRRD) requested the Health Effects Division (HED) to perform a review of a developmental neurotoxicity study in rats (MRID 45630301) for methyl parathion. The action was successfully completed, and the conclusions of the study are reported here.

I. CONCLUSIONS

The Registration Action Branch I (RAB 1) has reviewed the developmental neurotoxicity study in rats (MRID 45630301) for methyl parathion. The study 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 submitted study does satisfy the re-registration requirement of a developmental neurotoxicity study for methyl parathion.

1 of 1

Primary EPA Reviewer: Robert J. Mitkus, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)
EPA Work Assignment Manager: P. V Shah, Ph.D.
Toxicology Branch, Health Effects Division (7509C)

Signature: Robert J. Mitkus
Date 9/21/05
Signature:
Date 10/5/05

Template version 11/01

DATA EVALUATION RECORD

TXR#: 0050608

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

PC CODE: 053501

DP BARCODE: D281892
SUBMISSION NO.: S612773

TEST MATERIAL (PURITY): Methyl parathion (96.8% a.i.)

SYNONYMS: Phosphorothioic acid, *O*, *O*-dimethyl *O*-(4-nitrophenyl) ester

CITATION: Beyrouty, P. (2002) A developmental neurotoxicity study of orally administered methyl parathion in the rat. ClinTrials BioResearch Ltd., 87 Senneville Road, Senneville, Quebec, Canada H9X 3R3. Laboratory Project ID. 97574, March 1, 2002. MRID 45630301. Unpublished.

SPONSORS: Cheminova A/S, P.O. Box 9, DK-7620 Lemvig, Denmark
Griffin LLC, P.O. Box 1847, Valdosta, GA 31603-1847

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRID 45630301), methyl parathion (96.8% a.i., lot # 621-BSe-20A) was administered to 32 pregnant female Crl:CD® (SD) IGS BR rats/group by gavage at doses of 0, 0.03, 0.30, or 0.60 mg/kg/day from gestation day 6 through postnatal day 10. The resulting offspring were dosed on lactation days 11-21. A Functional Observational Battery (FOB) was performed on all dams on gestation days 12 and 18 and on lactation days 4 and 10. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Randomly-selected pups (at least 10/sex/dose) were selected for either the FOB on lactation days 4, 11, and 21, neuropathological evaluation and brain weights on days 11, 22, and 70, motor activity on days 13, 17, 21, and 70, passive avoidance on days 23 and 24, auditory startle habituation on days 23 and 60, or water maze testing on days 58-63. Pup physical development was assessed by bodyweight; sexual maturation was assessed by age at vaginal opening for females and age at completion of balano-preputial separation for males. No treatment-related effects were seen in the dams on survival, body weight, body weight gain, food consumption, clinical signs, or reproductive performances.

DATA EVALUATION RECORD

METHYL PARATHION/ 053501

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 45630301

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 02-34

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Disclaimer

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

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The maternal NOAEL is 0.60 mg/kg/day, the highest dose tested. The maternal LOAEL is not established.

Treatment had no adverse effects on the offspring survival, clinical signs, developmental landmarks, body weight, body weight gain, motor activity, auditory startle reflex, learning and memory, brain weights, brain morphology or neuropathology. During the period of direct dosing, 12 pups (5 males and 7 females) from 5 litters at the high dose exhibited tremors post-dosing only on Day 13.

The offspring LOAEL is 0.60 mg/kg/day, based on tremors observed on PND 13. The offspring NOAEL is 0.30 mg/kg/day.

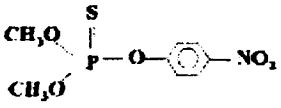
This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes; however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive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

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Methyl parathion

Description:	brown solid
Lot/Batch #:	621-BSe-20A
Purity:	96.8% a.i.
Compound Stability:	expiration date February 2002
CAS # of TGAI:	298-00-0
Molecular Structure:	

2. **Vehicle and/or positive control:** The vehicle was corn oil (Sigma Chemical Co., Lot # 107H1649). No positive control was used in this study.

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

Species:	Rat	
Strain:	Crl:CD® (SD) IGS BR	
Age at study initiation:	80-90 days old	
Wt. at study initiation:	244-324 g	
Source:	Charles River Canada, Inc., St. Constant, Quebec	
Housing:	In stainless steel mesh bottomed cages until GD 18 then transferred to solid bottomed cages with corn cob bedding. Pups were housed in groups of 2-3/sex for the first week after weaning, then transferred to individual housing.	
Diet:	PMI Certified Rodent Chow 5002, *ad libitum*	
Water:	reverse osmosis and ultraviolet sterilized tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	50±20%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 12 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: February 11, 2001; End: May 19, 2001

2. **Study schedule:** The females were mated and assigned to study groups of 32 each. The test substance was administered to the maternal animals from gestation day (GD) 6 through lactation day 10. Pups were weaned on postnatal day 22, after which time the dams were killed. F_1 animals remained on study up to approximately postnatal day 70.

3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily for the presence of sperm in a vaginal smear. The day that evidence of mating was found was designated GD 0.

4. **Animal assignment:** Mated females were allocated to groups using a computer-based random number generator (Table 1). Randomly-selected pups (at least 10/sex/dose) were selected for either an observational battery on lactation days 4, 11, and 21, neuropathological evaluation and brain weights on days 11 and 22, or motor activity on days 13, 17, and 21. At or shortly before weaning, up to 3 pups/sex/litter were randomly selected to provide the F_1 generation. The same animals were used, where possible, for both weanling and adult FOB, motor activity, or auditory startle tests, respectively.

TABLE 1. Study design

Experimental parameter	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Maternal animals				
FOB (prior to initiation, GD 12, 18; PND 4, 10)	All animals (n = 32)			
Offspring				
Motor activity (PND 13, 17, 21, 60)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 23, 60)	10/sex	10/sex	10/sex	10/sex
Passive Avoidance (PND 23, 24)	10/sex	10/sex	10/sex	10/sex
Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)[a]	10/sex	10/sex	10/sex	10/sex
Water maze (PND 58-62)	10/sex	10/sex	10/sex	10/sex
Brain Weight (PND 11, 22, 70)	10/sex	10/sex	10/sex	10/sex
Neuropathology (PND 11, 22, 70)	10/sex	10/sex	10/sex	10/sex

[a]A detailed clinical examination was performed on PND 22, rather than on PND 21. Detailed clinical observations on PNDs 35, 45, and 60 could not be verified.

5. **Dose selection rationale:** Dose levels were chosen based on the results from a range-finging study (Project # 97530; MRID 45631501) and a study of cholinesterase activity in dams, fetuses, and pups (Project # 97558; MRID 45646501). In the range-finding study, rats were administered 0, 0.1, 1.0, or 2.0 mg/kg/day by oral gavage either on GD 6-20 or GD 6 through lactation day 10. Offspring from dams allowed to litter were treated on lactation days 11-21. High-dose dams showed clinical signs of neurotoxicity and had decreases in body weight gains and food consumption throughout gestation. Pups from the high-dose dams had decreased survival throughout lactation and clinical signs of neurotoxicity were observed in mid- and high-dose pups after the initiation of direct treatment. Eye lesions were observed in a small number of mid- and high-dose pups. In dams and fetuses on GD 20 and in pups on lactation day 21, substantial (>50%) inhibition of cholinesterase (ChE) activity was noted in all compartments (plasma, RBCs, and brain) for the mid- and high-dose groups. On lactation day 21, plasma ChE activity was slightly reduced (by 16%) in the low dose male pups only.

This response was considered non-adverse by the study authors, and a NOAEL of 0.1 mg/kg/day was identified based on both clinical findings and ChE inhibition.

In the study of cholinesterase activity (MRID 45646501), pregnant rats were administered 0, 0.03, 0.30, 0.60 mg/kg/day methyl parathion on either GD 6-20 or GD 6 through lactation day 10, and the resulting offspring were treated from lactation day 11-21 ("Phase I"). Additional groups included young adult rats treated at similar levels for 1 or 11 days ("Phase III") and neonates from untreated dams administered 0, 0.03, 0.11, 0.30, or 1.00 mg/kg on lactation day 11 ("Phase II"). Cholinesterase activities in plasma, RBC, and brain were measured from all dams and pups. Significant inhibition was found in all three compartments following direct treatment at doses of ≥0.30 mg/kg/day. No clinical signs, effects on body weights or food consumption, or effects on maternal performance were observed. The NOAELs were 0.03 mg/kg/day for dams and 0.30 mg/kg/day for fetuses following dosing of the dams on GD 6 through lactation day 10 ("Phase I"); 0.03 mg/kg/day for pups following dosing on lactation days 11-21 ("Phase I"); 0.11 mg/kg for pups and 0.60 mg/kg for young adults following a single administration ("Phases II" and "III," respectively); and 0.03 mg/kg/day for young adults following 11 days of dosing ("Phase III").

Therefore, doses for the main study were chosen at levels known to cause significant cholinesterase inhibition, but which should not compromise neurotoxicity testing.

6. **Dosage administration:** The test article was administered by gavage to dams on GD 6 through lactation day 10 and to the resulting offspring on lactation days 11-21. A dose volume of 5 ml/kg/day was administered to all animals and was formulated based on each animal's most recent body weight.

7. **Dosage preparation and analysis:** Dosage formulations were prepared weekly. Solutions for the high-dose group were prepared by mixing the appropriate amounts of the test article with corn oil. The solution was mixed on a stir-plate with a magnetic stir bar until dissolved. The solutions for the lower dose groups were prepared by serial dilution. No correction was made for purity of the test article. The dose solutions were stirred continuously throughout use. Samples for concentration analysis were taken from solutions prepared at the beginning, middle, and end of the study.

Results:

Homogeneity analysis: Not reported.

Stability analysis: Not reported.

Concentration analysis: Concentrations of the dosing solutions prepared for days 1, 15, and 36 ranged from 91.3% to 107% of nominal.

C. OBSERVATIONS

1. In-life observations:

a. Maternal animals: All animals were checked twice daily for mortality and clinical signs of toxicity. A complete detailed examination was performed on the days of body weight assessment. A detailed examination was made at approximately one hour after dosing. Findings observed post-dosing were re-evaluated the next day prior to dosing to assess reversibility. Abnormalities in nesting or nursing behavior were recorded. Beginning on GD 20, the females were observed 3 times/day for signs of parturition. Where possible, when parturition was observed, the times of onset and completion of parturition were recorded and any sign of dystocia was noted.

The FOB was conducted on all dams prior to the initiation of treatment and then before daily dosing on GD 12 and 18 and on lactation days 4 and 10. Testing was done by trained technicians who were blind to the treatment group. The following functional observations were recorded:

	Functional Observations–Maternal animals
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

The tests were conducted in the animal housing room where environmental conditions were monitored. Odors in the room were minimized by maintaining adequate air changes and by cleaning of equipment, as necessary.

Individual maternal body weights were measured on GD 0, 3, 6, 9, 12, 15, 18, and 20 and on lactation days 0, 4, 7, 11, 13, 17, and 21. Maternal food consumption was recorded for GD 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20 and for lactation days 0-4, 4-7, 7-11, 11-13, 13-17, and 17-21.

b. Offspring:

1) Litter observations: The day of completion of parturition was designated as lactation day 0; pups were examined for malformations, sexed, and the numbers alive and dead were recorded. Live pups were weighed; malformed pups were euthanized and along with any dead pups were

examined internally or placed in Bouin's solution for subsequent examination. The general condition of the pups was evaluated daily throughout lactation. A detailed examination was made and body weights were assessed on lactation days 4, 7, 11, 13, 17, and 22. In addition, a detailed examination was made at approximately one hour post-dosing to dams and approximately 0.5-1 hour after direct dosing of the pups. Findings observed post-dosing were re-evaluated the next day prior to dosing to assess reversibility. Pups found dead between lactation days 0 and 7 were immediately given an internal examination or were stored in Bouin's solution for subsequent examination. Pups found dead between lactation days 8 and 21 were given a complete necropsy.

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

2) **Developmental landmarks:** Beginning on postnatal day 34, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 26, female offspring were examined daily for vaginal opening.

3) **Postweaning observations:** After weaning on postnatal day 22, offspring were examined twice daily for mortality and signs of ill health. A complete detailed examination was performed weekly. Individual body weights were recorded weekly, and animals selected for perfusion or brain weights were weighed on the day of sacrifice.

4) **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report. One male and one female per litter (at least 10/sex/group) were randomly selected for each of the following tests. For each specific behavioral test, the same animal was used across time, if possible.

   a) **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, selected offspring were examined outside the home cage in an FOB assessment by the same observers, whenever possible, who were also blind to the treatment groups. On postnatal days (PND) 4 and 11, the animals were not evaluated via handling for lacrimation, pupil size, salivation, urinary staining, diarrhea, or body tone, as required by EPA guideline. In addition, observations in the FOB arena for abnormalities in gait, palpebral closure, and defecation/urination did not take place on PND 4 and 11. Last, evidence of piloerection, tremors, twitches, and convulsions was also not collected from pups on PND 4. No explanation was provided for any of these deficiencies. The same animals were used for pre- and post-weaning FOB and motor activity evaluations, respectively. The tests were conducted in the animal housing room where environmental conditions were monitored. Odors in the room were minimized by maintaining adequate air changes and by cleaning of equipment, as necessary.

	FUNCTIONAL OBSERVATIONS- Offspring
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

b) **Motor activity testing:** Motor activity was evaluated on days 13, 17, 21, and 60. Animals were placed individually in figure-eight mazes and were continuously monitored over a 1-hour period. An automated activity monitoring system collected data over successive 10-minute intervals by recording infra-red light source break frequency within the maze. Sound levels in the room were kept constant at 70 dBA using exterior white noise generation. Temperature and humidity were monitored, and illumination in the room was approximately 600-1000 Lux. The same animals were used for pre- and post-weaning FOB and motor activity evaluations, respectively. No distinction was made between motor and locomotor activity; results were presented as both total and mean activity counts for each interval.

c) **Auditory startle habituation:** Auditory startle reflex habituation was performed on postnatal days 23 and 60. Animals were given a 4-minute acclimation period, and then the startle response was measured in 50 identical trials at a sound level of 120 dBA with an 8-second interval between trials.

d) **Learning and memory testing:**

Passive Avoidance: On postnatal day 23, learning and short- and long-term retention were assessed in a passive avoidance test. Each animal was placed into the illuminated side of a two-compartment rodent shuttle cage and the time elapsed before crossing to the darkened side was recorded. When the animal crossed to the darkened side it received a foot shock of 1.5 milliamps for 1 second (conditioning trial). After approximately 30 seconds this trial was repeated a second time, and if necessary a third time. Animals remaining on the lighted side for 2 minutes were considered to have met criteria for the conditioning test. One hour after the conditioning trial(s), those animals which achieved criteria were tested for step-through latency. No shock was delivered to those that entered the darkened side. On postnatal day 24 (at least 25 hours after the conditioning trial), those animals that achieved criteria at the one-hour trial were tested again with step-through latency recorded for a 2-minute period. No shock was delivered to those animals that entered the darkened side.

Cincinnati Water Maze: Learning and memory testing using the water maze ensued between postnatal days 58 and 62. The animal's ability to swim was assessed initially by measuring the time to swim a straight channel; this was repeated four times. On the next day, learning and memory test were conducted using a Cincinnati water maze consisting of two paths. On the first day of testing, each animal was tested twice (at least 10 minutes apart) by measuring the time to complete the first path (Path A). This was repeated on two additional consecutive days (second trial on day 1 and first trial on day 2 were at least 25 hours apart) using the same path. The same paradigm was repeated using the second path (Path B). At least one day separated testing of the two paths. Any abnormal swimming ability was recorded.

5) **Cholinesterase determination:** Cholinesterase activity in blood and brain were not measured as part of the current study. Detailed inhibition studies in dams, fetuses, pups, and adults were submitted as a separate study which included a dosing regime similar to the current study (Project # 97588, MRID 45646501).

2. **Postmortem observations:**

a. **Maternal animals:** Animals found dead or sacrificed during the study were given a complete necropsy. Maternal animals surviving to scheduled termination were sacrificed by carbon dioxide asphyxiation followed by exsanguination from the abdominal aorta on postnatal day 22 or 23. Dams were subjected to gross necropsy, and the number of implantation scars was recorded. Females that mated but did not deliver were killed on day 26 *post coitum* and reproductive tracts were examined for abnormalities. The uterus of any animal judged to be non-pregnant was stained with 10% ammonium sulfide solution and examined for implantation sites. Dams with whole litter loss were sacrificed, the number of implantation sites were recorded, the mammary tissue was examined, and a sample was retained. From each animal, the mammary glands, ovaries, uterus, vagina, and any abnormal tissues were fixed in 10% neutral buffered formalin, but not examined.

b. **Offspring:** Pups dying on or before lactation day 7 were immediately given a complete external and internal examination or placed in Bouin's solution for subsequent examination. Pups dying between lactation days 8-22 and weanlings found dead were given a complete gross examination as soon as possible. Weanlings not selected for behavioral testing or sacrificed during the study were euthanized by carbon dioxide asphyxiation followed by exsanguination from the abdominal aorta and subjected to gross necropsy.

The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 11, 22, or 70. These animals were subjected to postmortem examinations as described below.

On postnatal day 11, 10 pups/sex/group (plus spares) were selected from different litters for brain measurements. Animals were sacrificed by carbon dioxide asphyxiation followed by

exsanguination from the abdominal aorta. The calvarium was removed to expose the brain, and the entire head was immersion fixed in Bouin's fluid for approximately 4 hours and then transferred to 10% buffered formalin for 6 or 7 days. The spinal cord was retained in neutral-buffered 10% formalin. The remaining carcass was discarded without further examination.

On postnatal day 22, 10 pups/sex/group (plus spares) from different litters were deeply anesthetized by intraperitoneal injection of sodium pentobarbital and perfused via the left ventricle with a heparinized sodium chloride solutions followed by fixation with gluteraldehyde and paraformaldehyde. The animals were skinned, and the thoracic and abdominal organs were removed and placed in 10% neutral-buffered formalin for possible future examination. The calvarium was removed to expose the brain and the entire head was placed in 10% buffered formalin for 6 or 7 days. The remaining carcass containing the spinal cord and limbs was placed in 10% neutral-buffered formalin.

For pups killed on lactation day 11 or 22, brain weight (with olfactory bulbs) and length measurements (without olfactory bulbs) of anterior cerebrum to posterior cerebrum and anterior cerebrum to posterior cerebellum together with cerebral and cerebellar maximal coronal width measurements, were recorded prior to trimming. Brains from these animals were embedded in paraffin wax, sectioned at 6 μm and stained with hematoxylin and eosin. Five sections from 22-day old control and high-dose animals were examined microscopically for histopathology and morphometry. However, the specific histopathological endpoints were not listed in the study protocol. Because of this, it is unclear if the specific microscopic neuropathological alterations outlined in EPA Guideline OPPTS 870.6300 were evaluated. Morphometry measurements were undertaken using a BIOQUANT/TCW® image analysis system. Estimates of the thickness of major layers at representative locations within the center of the cerebrum (neocortex, hippocampus, corpus callosum) and cerebellum were determined.

On approximately postnatal day 70, 10 animals/sex/group were euthanized by carbon dioxide asphyxiation, underwent a gross necropsy, and the brains were removed and weighed (fresh weight) and discarded. Another 10 rats/sex/dose were deeply anesthetized by intraperitoneal injection of sodium pentobarbital and perfused via the left ventricle with a heparinized sodium chloride solution followed by fixation with gluteraldehyde and paraformaldehyde. The animals were skinned, and the thoracic and abdominal organs were removed and placed in 10% neutral-buffered formalin for possible future examination. The calvarium was removed to expose the brain, and the entire head was placed in 10% buffered formalin for 6 or 7 days. The remaining carcass containing the spinal cord and limbs was placed in 10% neutral-buffered formalin. The brains of all animals and all tissues from the control and high-dose animals were processed to the slide stage. Brain weight and length (anterior cerebrum to posterior cerebrum and anterior cerebrum to posterior cerebellum together with cerebral and cerebellar maximal coronal width) measurements were recorded prior to trimming. Nervous system tissues of all animals were examined grossly at the time of sampling.

Central and peripheral nervous tissues and skeletal muscle were dissected and preserved in paraffin (brain, eyes, spinal cord, skeletal muscle, and gross CNS lesions only) or plastic (e.g., sural, sciatic, and tibial nerves; lumbar and cervical roots). Tissues from all dose groups were embedded; however, only control and high-dose tissues were examined unless effects warranted examination of low- and mid-dose samples. Paraffin-embedded tissues were sectioned at 6 µm and stained with hematoxylin and eosin. Plastic-embedded tissues were sectioned at 0.5 µm and stained with borate-buffered 1% toluidine blue. However, the specific histopathological endpoints were not listed in the study protocol. Because of this, it is unclear if the specific microscopic neuropathological alterations outlined in EPA Guideline OPPTS 870.6300 were evaluated. Morphometry measurements were undertaken using a BIOQUANT/TCW® image analysis system. Estimates of the thickness of major layers at representative locations within the center of the cerebrum (hippocampus, corpus callosum) and cerebellum were determined.

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

X	CENTRAL NERVOUS SYSTEM
	BRAIN
x	**Coronal sections:** olfactory bulbs, forebrain (through septum), cerebrum (through hypothalamus), midbrain
x	**Mid-sagittal sections**: cerebellum (incl. pons and medulla oblongata)
	SPINAL CORD
x	Cervical **
x	Thoracic **
x	Lumbar **
	OTHER
x	Gasserian Ganglion*
	Trigeminal nerves
x	Optic nerve
x	Eyes

X	PERIPHERAL NERVOUS SYSTEM
	SCIATIC NERVE
x	Mid-thigh**
x	Sciatic Notch**
	OTHER
x	Sural Nerve (at knee)**
x	Tibial Nerve (at knee)**
	Peroneal Nerve
x	Lumbar dorsal root ganglion*
x	Lumbar dorsal root fibers*
x	Lumbar ventral root fibers**
x	Cervical dorsal root ganglion*
x	Cervical dorsal root fibers*
x	Cervical ventral root fibers*
x	gastrocnemius muscle **

* cross-section
** longitudinal and cross-sections

D. DATA ANALYSIS

1. **Statistical analyses:** Group means for maternal body weights, body weight gains, and food consumption and pup body weights were analyzed using the parametric one way analysis of variance (ANOVA), followed by Dunnett's test if significance was identified with the ANOVA. Numbers of live pups and implantation sites, post-implantation loss, gestation length, and viability, survival and lactation indices were analyzed by the non-parametric

Kruskall-Wallis and Mann-Whitney "U" tests. The incidence of dead pups and malformed pups were analyzed using the chi-square test or Fisher's exact probability test.

Physical development data were analyzed by Kruskal-Wallis and Mann- Whitney "U" tests. Motor activity counts were analyzed using repeated measures analysis, and graphical presentation of the data was made. Startle habituation data were averaged for each set of 10 teials and then analyzed using repeated measures analysis. Observational data were compared between groups using a Fisher's exact test, where appropriate. Latency times for passive avoidance and escape times for the Cincinnati water maze were analyzed by ANOVA followed by Dunnett's test. The incidence for passive avoidance data was analyzed using Fisher's exact probability test, and the errors for the water maze were analyzed using the Kruskal-Wallis and Mann-Whitney "U" tests.

Group variances for morphometry data were compared using Bartlett's test. If not significant, ANOVA was used followed by Dunnett's test. When the differences in group variance were significant, the Kruskall-Wallis test was performed followed by Dunn's or Wilcoxon's test.

2. **Indices:**

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

Post-implantation loss = [(No. implantation sites - No. live pups at birth)/No. implantation sites] × 100

Pregnancy rate = (No. rats pregnant/No. rats mated) × 100

Gestation index = (No. live litters born/No. pregnant rats) × 100

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

Viability index = (No. of live pups on PND 4 pre-cull/No. live pups on PND 0) × 100

Survival index = (No. of live pups on PND 7 or 14/No. live pups on PND 4 or 11 post-cull) × 100

Lactation index = (No. live pups on Day 21/No. live pups on PND 4 or 11 post cull) × 100

3. **Positive and historical control data:** Positive and historical control data were not presented, and no references to positive control studies were given in the study. A statement was made that "trained technicians" conducted the FOB. However, positive control (PC) data were subsequently submitted to the EPA and are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** One high-dose dam was sacrificed on GD 22 due to dystocia; clinical findings in this animal included reduced body temperature, decreased activity, flaccid muscle tone, hunched posture, weakness, dehydration, ocular discharge, and fur staining. In addition, one mid-dose dam was sacrificed on lactation day 9 following complete litter loss due to a "technical error/malfunction" (no additional information reported). Both of these deaths were considered incidental to treatment.
Salivation or wet muzzle/lower jaw were observed post dosing on single occasions in 4-5 high-dose dams compared with a few (1-3) animals in the control, low-, or mid-dose group. Other clinical findings common to both treated and control animals included fur loss and scabs.

   No dose- or treatment-related differences in functional parameters were observed during gestation or lactation. However, on gestation day 12, mid-dose dams exhibited a decrease ($p \leq 0.05$) in defecation, while low-dose dams displayed an increase ($p \leq 0.05$) in "slight" urination. These effects are not biologically significant and are attributed to inter-group variation.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 2. No dose- or treatment-related differences in absolute body weights, body weight gains, or food consumption were observed between the treated and control groups during gestation or lactation. Body weight gain by the low-dose dams was significantly ($p \leq 0.05$) greater than that of the control group for the lactation day 13-17 interval; this is considered incidental to treatment.

TABLE 2. Selected mean (±SD) maternal body weight and food consumption data[a]

Observations/study interval	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Gestation (n = 30-32)				
Body wt. Gestation day 0 (g)	257.1 ± 12.3	256.1 ± 10.1	256.3 ± 12.0	254.2 ± 12.7
Body wt. Gestation day 6 (g)	282.7 ± 15.2	282.8 ± 13.3	280.9 ± 14.6	282.1 ± 15.9
Body wt. Gestation day 15 (g)	320.4 ± 19.8	322.9 ± 15.5	322.0 ± 17.5	322.1 ± 16.0
Body wt. Gestation day 20 (g)	386.6 ± 23.8	388.5 ± 20.8	384.5 ± 25.2	383.3 ± 22.8
Wt. gain Gestation days 6-9 (g)	4.9 ± 6.2	4.3 ± 6.7	5.5 ± 8.1	5.2 ± 6.9
Wt. gain Gestation days 12-15 (g)	16.1 ± 9.9	18.0 ± 5.4	18.8 ± 6.6	16.7 ± 5.6
Wt. gain Gestation days 18-20 (g)	26.8 ± 5.9	25.8 ± 6.9	24.2 ± 7.1	23.1 ± 10.3
Food consumption gestation days 6-9 (g/animal)	59.4 ± 8.1	59.9 ± 6.4	58.9 ± 8.7	62.3 ± 9.4
Food consumption gestation days 12-15 (g/animal)	68.1 ± 8.5	69.4 ± 7.6	69.8 ± 9.0	69.2 ± 8.7

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. Pregnancy rate, gestation index, length of gestation, number of implantation sites, and post-implantation loss were similar between the treated and control groups.

TABLE 3. Reproductive performance [a]

Observation	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Number Mated	32	32	32	32
Number Pregnant	30	32	31	32
Pregnancy Rate (%)	93.8	100	96.9	100
Gestation Index (%)	100	100	96.8	96.9
Gestation Length (days)	22.0 ± 0.32	21.9 ± 0.30	21.9 ± 0.25	21.9 ± 0.30
No. Implantation Sites	16.3 ± 1.90	16.0 ± 2.24	15.9 ± 3.23	15.9 ± 2.26
Post-implantation Loss (%)	4.8	6.8	9.5	8.3

[a]Data obtained from Table 10, pp. 99-100, MRID 45630301.

4. **Maternal postmortem results:** No treatment-related gross lesions were found in any female at necropsy.

TABLE 2. Selected mean (±SD) maternal body weight and food consumption data[a]				
Observations/study interval	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Food consumption gestation days 18-20 (g/animal)	41.4 ± 5.6	40.3 ± 5.1	38.9 ± 4.2	40.4 ± 8.7
Lactation (n = 29-32)				
Body wt. lactation day 0 (g)	294.9 ± 17.9	297.4 ± 18.6	296.5 ± 18.7	297.4 ± 18.5
Body wt. lactation day 4 (g)	310.1 ± 16.7	311.5 ± 16.5	309.1 ± 16.2	306.5 ± 15.7
Body wt. lactation day 7 (g)	314.6 ± 17.6	317.9 ± 15.8	315.0 ± 16.8	314.4 ± 17.0
Body wt. lactation day 13 (g)	335.2 ± 19.1	332.6 ± 19.9	335.8 ± 21.1	331.6 ± 15.6
Body wt. lactation day 21(g)	316.0 ± 18.9	319.2 ± 17.3	319.6 ± 20.9	317.2 ± 19.8
Food consumption lactation days 0-4 (g/animal)	118.1 ± 20.3	116.6 ± 18.7	114.5 ± 16.1	118.8 ± 18.9
Food consumption lactation days 7-11 (g/animal)	198.9 ± 20.3	194.6 ± 19.1	193.1 ± 17.1	204.8 ± 16.0
Food consumption lactation days 17-21 (g/animal)	257.9 ± 39.2	255.6 ± 26.2	263.6 ± 26.1	259.2 ± 23.7

[a]Data obtained from Tables 2-4, pp. 76-78, Tables 6 & 7 pp. 80-81, and Appendices 2-4 & 6-7, pp. 243-266 & 276-290, MRID 45630301.

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability (survival) during lactation are summarized in Table 4. No treatment-related effects were observed on the number of litters, live litter size, number of stillborn pups, sex ratios at birth, or pup survival. No treatment-related clinical signs of toxicity were observed in any pup during lactation days 0-10. During the period of direct dosing, a total of 12 high-dose pups (5 males and 7 females) from 5 litters showed tremors post-dosing on day 13 only. In addition, 3, 3, and 6 pups from the low-, mid-, and high-dose groups, respectively, had post-dosing salivation on 1-3 days during the treatment period.

   During the post weaning interval, one low-dose male was found dead on PND 24 with no prior clinical signs, and one control male was sacrificed due to poor condition on PND 40; these deaths were considered incidental to treatment. All remaining animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal during the post-weaning interval.

TABLE 4. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Number of Litters	30	32	30	31
Sex ratio at birth (% male)	50.2	49.2	53.0	52.3
Mean no. dead pups at birth	0.10 ± 0.305	0.19 ± 0.471	0.27 ± 0.583	0.26 ± 0.575
Mean No. of viable pups:				
Day 0	15.5 ± 2.06	14.8 ± 2.29	15.4 ± 1.90	14.5 ± 1.96
Day 4 [b]	15.4 ± 2.04	14.7 ± 2.19	15.1 ± 1.82	14.5 ± 1.98
Day 4 [c]	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00
Day 21	6.9 ± 0.31	6.9 ± 0.25	6.9 ± 0.31	7.0 ± 0.18
Viability index day 4 (%)	99.2	98.8	98.1	99.3
Survival index day 7 (%)	100	100	100	100
Survival index day 14 (%)[d]	100	100	100	100
Lactation index day 21 (%)[d]	98.6	99.1	98.5	99.5

[a]Data obtained from Table 10-12, pp. 99-105, MRID 45630301.
[b]Before standardization (culling).
[c]After standardization (culling).
[d]Does not include one pup/litter culled on day 11.

2. **Body weight:** Selected pup body weights during lactation are given in Table 5. Weight gains for pups were not calculated by the study author. Absolute body weights were comparable at birth across all dose groups. Body weights were significantly ($p \leq 0.05$) greater than the controls on day 4 for the high-dose males and females and mid-dose females (post-cull only) and on day 7 for the high-dose females. These results may have been due to slightly lower body weights for the controls as a result of a slightly greater number of pups/litter prior to culling. Differences in body weights were not toxicologically significant and not dose-related.

TABLE 5. Mean (±SD) pre-weaning pup body weights (g) [a]

Postnatal Day	Dose (mg/kg/day)							
	0	0.03	0.30	0.60	0	0.03	0.30	0.60
	Males				Females			
0	6.2 ± 0.46	6.4 ± 0.48	6.1 ±0.37	6.4 ± 0.49	5.9 ± 0.40	6.1 ± 0.42	5.7 ± 0.39	6.1 ± 0.47
4 (precull)	9.5 ± 0.84	10.0 ±0.97	9.6 ±0.77	10.1* ± 0.87	9.2 ± 0.78	9.7 ± 0.87	9.2 ± 0.77	9.7* ± 0.88
4 (post cull)	9.6 ± 0.81	10.1 ±0.92	9.8 ±0.73	10.1* ± 0.88	9.3 ± 0.77	9.8* ± 0.82	9.4 ± 0.67	9.8* ± 0.88
11	25.5 ± 2.20	26.1 ±1.63	25.4 ± 1.53	26.2 ± 1.64	24.8 ± 2.02	25.4 ± 1.57	24.6 ± 1.48	25.6 ± 1.70
17	42.0 ± 3.62	42.6 ±2.79	42.1 ± 2.48	42.8 ± 3.47	40.8 ± 3.41	41.4 ± 2.92	40.6 ± 2.53	41.5 ± 2.96
22	60.6 ± 5.57	61.4 ±4.55	60.6 ± 4.02	61.3 ± 4.84	58.7 ± 5.00	59.6 ± 3.70	58.7 ± 3.79	58.5 ± 3.58

[a] Data obtained from Table14, pp. 109-112, MRID 45630301.
Significantly different from control *$p \leq .05$.

Selected mean post weaning offspring body weight data are presented in Table 6. Mean body weights for males and females were similar between the treated and control groups throughout the post-weaning interval. Food consumption was not measured.

TABLE 6. Mean (±SD) post-weaning pup body weights (g) [a]

Postnatal Day	Dose (mg/kg/day)							
	0	0.03	0.30	0.60	0	0.03	0.30	0.60
	Males				Females			
29	102.3 ± 7.59	105.2 ± 7.07	104.0 ± 6.86	105.52 ± 6.33	95.2 ± 6.63	96.4 ± 5.51	94.5 ± 5.79	95.3 ± 5.27
36	164.3 ± 13.96	166.0 ± 13.32	166.9 ± 11.24	166.6 ± 10.06	141.3 ± 7.01	142.4 ± 7.84	138.7 ± 8.30	139.9 ± 6.97
43	228.2 ± 19.24	229.5 ± 16.39	231.0 ± 14.86	231.3 ± 13.25	174.5 ± 10.65	177.5 ± 10.90	172.3 ± 10.35	175.6 ± 9.46
50	290.4 ± 24.07	291.3 ± 20.86	295.1 ± 18.19	293.2 ± 16.61	202.9 ± 13.53	204.8 ± 14.15	198.4 ± 13.06	202.0 ± 10.97
57	352.1 ± 26.89	349.4 ± 25.04	354.0 ± 23.29	352.6 ± 21.06	228.8 ± 15.43	232.3 ± 16.73	224.2 ± 16.77	229.3 ± 14.06
64	398.7 ± 29.50	392.3 ± 28.74	398.0 ± 27.86	396.7 ± 24.08	248.1 ± 16.56	253.2 ± 19.65	245.1 ± 20.30	249.8 ± 17.44
69	431.0 ± 33.14	424.5 ± 30.78	430.0 ± 30.56	427.9 ± 27.01	264.7 ± 18.95	268.7 ± 22.17	259.2 ± 22.09	264.9 ± 17.86

a Data obtained from Table26, pp. 164-167, MRID 45630301.

3. **Sexual maturation:** No treatment-related effects on the mean age for attainment of vaginal opening for females or preputial separation for males were found. The data are presented in Table 7.

TABLE 7. Mean (±SD) age of sexual maturation (days) [a]

Endpoint	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Preputial separation (males)	42.2 ± 1.30	42.1 ± 1.30	41.9 ± 1.38	41.6 ± 1.15
Vaginal opening (females)	32.2 ± 1.15	32.1 ± 0.99	32.2 ± 1.05	31.9 ± 1.04

a. Data obtained from Tables 27 & 28, pp. 168 & 169, respectivley, MRID 45630301.

4. **Behavioral assessments:**

a. **Functional observational battery:** As pointed out in section C.1.b.4.a above, not all parameters of the observational battery were measured on each day. However, for those that were, no treatment-related effects were observed at any dose level on any test day (PND 4, 11, 21, 35, 45, or 60). On PND 21 mid-dose females had significantly ($p \leq 0.05$) fewer fecal boli compared with the controls (0.0 vs 0.7 for the controls). This isolated finding was considered sporadic and incidental to treatment.

b. **Motor activity:** Mean total activity counts are presented in Table 8. No distinction was made between motor and locomotor activity, and results were presented in the report as both total and mean activity counts for each interval. No treatment-related overall or interval motor activity (MA) effects were noted. However, mean MA data are relatively noisy for PND 13, 17, and 21 with coefficients of variation often >100% across dose on PND 13 and 17. In both males and females, decreased motor activity was seen on PND 17 at 0.6 mg/kg/day (24% in males and 37% in females); however, since there was no consistent dose-response, these decreases could not be attributed to treatment. Habituation across successive 10-minute testing intervals was apparent by PND 21 for both males and females, because MA approached asymptotic levels by the last 20% of the session in the control groups on this day.

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

Test Day	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Males (n = 14-16)				
PND 13	82.9 ± 62.3	67.8 ± 99.6	131.3 ± 110.2	109.9 ± 127.6
PND 17	119.5 ± 124.3	159.4 ± 128.7	150.2 ± 152.8	91.1 ± 107.8
PND 21	122.8 ± 50.0	114.4 ± 67.9	125.1 ± 74.3	123.3 ± 78.7
PND 60	481.8 ± 131.8	460.9 ± 144.7	480.3 ± 152.9	427.8 ± 145.4
Females (n = 15-16)				
PND 13	104.6 ± 84.1	100.0 ± 96.9	102.7 ± 70.4	99.1 ± 78.8
PND 17	132.1 ± 123.6	161.1 ± 117.2	129.9 ± 106.2	82.8 ± 86.5
PND 21	105.5 ± 64.7	103.8 ± 53.7	114.7 ± 56.9	84.7 ± 43.4
PND 60	555.7 ± 117.8	532.9 ± 142.1	572.9 ± 123.0	557.4 ± 135.9

[a] Data obtained from Tables 16 & 30, pp. 125-130 & 188-189, respectively, MRID 45630301.

c. **Auditory startle reflex:** Data for startle at start, maximum startle, time of maximum startle, and average startle are given in Tables 9, 10, 11, and 12, respectively. No dose- or treatment-related differences were noted between the treated and control groups for any endpoint on either testing day. Maximum and average startle responses for the low-dose males were significantly ($p \leq 0.05$) increased on day 60 compared with the controls; this

change was considered incidental to treatment due to the lack of a dose-response. Habituation was seen over successive trial blocks in all groups on both days for maximum and average startle responses.

TABLE 9. Startle at start (mean voltage ± S.D.) [a]

	Trials	Dose (mg/kg/day)			
		0	0.03	0.30	0.60
		Males (n = 14-16)			
PND 23	1-10	3.35 ± 1.87	3.84 ± 1.56	3.39 ± 1.55	3.57 ± 1.79
	11-20	3.25 ± 1.28	3.40 ± 1.49	4.03 ± 2.08	2.93 ± 1.87
	21-30	3.13 ± 1.23	3.53 ± 1.68	2.64 ± 1.15	3.17 ± 1.48
	31-40	3.36 ± 1.51	3.15 ± 1.59	3.64 ± 1.76	2.83 ± 1.32
	41-50	4.14 ± 2.95	3.18 ± 1.84	3.00 ± 1.14	2.90 ± 1.31
PND 60	1-10	7.37 ± 5.12	7.88 ± 5.18	8.03 ± 6.53	7.81 ± 5.58
	11-20	6.80 ± 3.47	6.12 ± 4.10	8.10 ± 8.99	6.09 ± 2.71
	21-30	9.45 ± 6.87	8.73 ± 5.73	7.92 ± 4.79	6.45 ± 2.57
	31-40	8.46 ± 5.17	7.58 ± 3.88	8.01 ± 3.03	7.07 ± 3.96
	41-50	9.14 ± 6.64	9.48 ± 4.75	8.40 ± 4.44	7.32 ± 3.66
		Females (n = 15-16)			
PND 23	1-10	3.72 ± 1.49	3.84 ± 1.61	4.19 ± 1.94	3.86 ± 3.01
	11-20	3.60 ± 1.97	3.19 ± 1.57	3.66 ± 1.49	2.81 ± 1.40
	21-30	3.27 ± 1.50	3.19 ± 0.96	3.89 ± 1.69	3.19 ± 1.81
	31-40	3.53 ± 1.21	2.94 ± 1.29	3.37 ± 1.80	3.31 ± 1.43
	41-50	2.93 ± 1.03	3.70 ± 1.82	3.23 ± 1.41	3.70 ± 1.28
PND 60	1-10	7.48 ± 4.85	7.83 ± 4.03	7.31 ± 4.50	7.63 ± 4.64
	11-20	6.21 ± 3.58	7.77 ± 5.99	6.15 ± 4.13	7.01 ± 4.95
	21-30	7.73 ± 2.90	9.02 ± 3.11	7.31 ± 4.28	6.32 ± 2.87
	31-40	8.17 ± 3.48	10.03 ± 9.02	6.16 ± 3.59	7.84 ± 4.17
	41-50	7.87 ± 2.21	8.14 ± 3.40	7.40 ± 3.03	8.26 ± 5.38

[a]Data obtained from Tables 17 &31, pp. 131-138 &190-197, respectivlely, MRID 45630301.

TABLE 10. Maximum startle (mean voltage ± S.D.)[a]

	Trials	Dose (mg/kg/day)			
		0	0.03	0.30	0.60
		Males (n = 14-16)			
PND 23	1-10	347.15 ± 94.12	434.39 ± 162.34	433.32 ± 188.20	413.91 ± 110.87
	11-20	304.50 ± 87.36	352.40 ± 102.28	341.71 ± 99.85	351.84 ± 128.93
	21-30	261.94 ± 68.74	319.81 ± 113.20	299.41 ± 126.30	306.79 ± 120.28
	31-40	270.30 ± 96.54	292.59 ± 105.39	286.58 ± 101.80	292.63 ± 144.37
	41-50	268.77 ± 101.98	263.17 ± 120.12	288.32 ± 95.89	280.77 ± 94.28
PND 60	1-10	662.69 ± 185.05	907.46 ± 454.39*	600.07 ± 305.10	697.29 ± 333.17
	11-20	451.31 ± 213.90	756.08 ± 502.53	415.51 ± 270.78	405.91 ± 275.96
	21-30	388.35 ± 189.19	610.76 ± 339.49	346.31 ± 188.38	413.06 ± 266.69
	31-40	330.57 ± 155.86	548.81 ± 302.04	271.82 ± 119.50	379.18 ± 235.06
	41-50	347.13 ± 156.86	584.38 ± 382.06	331.66 ± 192.84	375.03 ± 226.07
		Females (n = 15-16)			
PND 23	1-10	436.24 ± 126.84	409.28 ± 148.71	353.73 ± 88.42	498.31 ± 139.69
	11-20	353.93 ± 136.74	344.69 ± 95.92	331.27 ± 94.26	434.73 ± 92.17
	21-30	320.05 ± 73.00	267.94 ± 106.36	300.88 ± 106.42	378.03 ± 101.22
	31-40	303.04 ± 102.74	264.76 ± 114.07	266.09 ± 103.12	336.61 ± 97.84
	41-50	290.47 ± 128.52	236.06 ± 68.50	273.57 ± 85.80	327.83 ± 103.71
PND 60	1-10	622.70 ± 367.01	677.38 ± 317.95	632.25 ± 454.23	653.95 ± 190.21
	11-20	458.15 ± 318.37	536.43 ± 284.72	420.45 ± 318.30	551.03 ± 301.14
	21-30	369.43 ± 272.24	416.96 ± 211.07	326.94 ± 277.32	408.37 ± 220.72
	31-40	391.43 ± 293.12	407.01 ± 232.62	324.44 ± 370.77	395.68 ± 214.38
	41-50	363.19 ± 266.10	376.95 ± 199.68	316.69 ± 287.78	338.28 ± 191.04

[a]Data obtained from Tables 17 &31, pp. 131-138 &190-197, respectively, MRID 45630301.
* Significantly different from control: $p \leq 0.05$.

TABLE 11. Time of maximum startle (mean msec ± S.D.) [a]

	Trials	Dose (mg/kg/day)			
		0	0.03	0.30	0.60
		Males (n = 14-16)			
PND 23	1-10	22.20 ± 1.89	25.03 ± 5.92	24.19 ± 4.07	21.51 ± 2.97
	11-20	20.09 ± 1.54	20.63 ± 3.42	21.75 ± 3.12	19.86 ± 1.87
	21-30	19.82 ± 1.72	21.66 ± 3.56	20.74 ± 2.06	19.59 ± 1.47
	31-40	19.95 ± 1.69	21.49 ± 4.84	20.81 ± 2.26	19.54 ± 1.76
	41-50	20.91 ± 1.81	20.53 ± 2.07	20.03 ± 2.23	20.03 ± 1.51
PND 60	1-10	29.37 ± 3.65	30.23 ± 6.21	30.71 ± 4.45	31.25 ± 5.52
	11-20	26.99 ± 3.29	27.41 ± 4.88	26.41 ± 3.14	31.38 ± 7.75
	21-30	26.95 ± 3.50	26.09 ± 4.53	28.39 ± 3.65	30.31 ± 5.61
	31-40	27.92 ± 4.57	26.38 ± 3.81	29.86 ± 4.12	30.43 ± 5.39
	41-50	30.33 ± 4.84	28.08 ± 6.22	30.33 ± 6.30	28.44 ± 4.54
		Females (n = 15-16)			
PND 23	1-10	25.87 ± 5.29	22.73 ± 3.75	22.23 ± 3.11	24.76 ± 5.42
	11-20	20.67 ± 2.75	20.56 ± 2.12	21.33 ± 2.24	19.73 ± 1.81
	21-30	21.27 ± 3.63	20.34 ± 2.11	20.35 ± 2.28	19.56 ± 1.56
	31-40	20.86 ± 2.93	20.85 ± 3.16	19.98 ± 1.64	19.43 ± 1.94
	41-50	20.75 ± 3.09	20.83 ± 2.39	20.10 ± 2.21	20.82 ± 2.79
PND 60	1-10	30.89 ± 4.30	28.29 ± 3.41	30.95 ± 4.59	29.33 ± 3.69
	11-20	27.81 ± 3.25	26.85 ± 3.74	29.40 ± 4.31	27.24 ± 3.53
	21-30	29.61 ± 3.43	27.50 ± 3.64	29.53 ± 4.86	27.18 ± 3.58
	31-40	28.89 ± 4.57	27.91 ± 3.35	29.40 ± 5.35	27.45 ± 4.76
	41-50	29.30 ± 3.96	27.83 ± 3.49	28.91 ± 4.05	27.48 ± 3.24

[a]Data obtained from Tables 17 &31, pp. 131-138 &190-197, respectively, MRID 45630301.

TABLE 12. Average startle (mean voltage ± S.D.) [a]

	Trials	Dose (mg/kg/day)			
		0	0.03	0.30	0.60
		Males (n = 14-16)			
PND 23	1-10	61.44 ± 15.88	78.18 ± 31.15	79.59 ± 37.52	69.84 ± 18.01
	11-20	53.61 ± 16.80	61.43 ± 15.49	60.54 ± 18.63	58.88 ± 18.48
	21-30	48.07 ± 13.84	56.36 ± 17.26	53.03 ± 21.46	53.63 ± 18.48
	31-40	47.93 ± 17.24	51.79 ± 17.08	52.84 ± 20.11	51.63 ± 21.28
	41-50	48.31 ± 16.02	45.31 ± 16.86	54.32 ± 19.63	52.31 ± 15.87
PND 60	1-10	147.05 ± 39.58	205.96 ± 108.96*	137.03 ± 73.11	161.98 ± 73.12
	11-20	95.17 ± 42.97	160.23 ± 93.54	91.18 ± 55.19	92.85 ± 58.51
	21-30	82.97 ± 39.44	131.23 ± 70.96	75.31 ± 37.76	90.47 ± 50.71
	31-40	70.35 ± 30.86	117.79 ± 63.94	59.81 ± 27.62	83.43 ± 45.23
	41-50	74.27 ± 30.74	124.93 ± 81.31	74.63 ± 42.26	80.56 ± 41.76
		Females (n = 15-16)			
PND 23	1-10	81.26 ± 26.35	72.10 ± 27.06	62.49 ± 18.11	91.32 ± 29.64
	11-20	62.07 ± 20.64	60.72 ± 17.42	57.06 ± 16.64	73.17 ± 14.75
	21-30	56.95 ± 14.19	47.88 ± 17.03	52.63 ± 18.89	64.11 ± 16.25
	31-40	55.33 ± 20.47	48.19 ± 19.25	47.65 ± 18.11	59.12 ± 15.13
	41-50	51.15 ± 19.32	44.82 ± 12.85	48.82 ± 16.24	57.26 ± 15.19
PND 60	1-10	137.98 ± 78.73	141.71 ± 64.55	131.94 ± 92.61	143.07 ± 43.69
	11-20	94.69 ± 56.60	109.14 ± 52.89	87.58 ± 62.81	115.01 ± 59.99
	21-30	79.35 ± 54.55	86.49 ± 43.39	68.98 ± 58.60	86.76 ± 46.54
	31-40	84.90 ± 59.52	86.98 ± 47.38	68.73 ± 70.31	81.64 ± 42.67
	41-50	76.45 ± 51.54	77.48 ± 39.87	67.67 ± 55.95	71.42 ± 38.00

[a]Data obtained from Tables 17 &31, pp. 131-138 &190-197, respectively, MRID 45630301.
* Significantly different from control: $p \leq 0.05$.

d. Learning and memory testing:

Passive Avoidance: No treatment-related effects on acquisition and memory of the avoidance task were found. Data are summarized in Table 13.

TABLE 13. Passive avoidance performance at PND 23/24 [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.03	0.30	0.60
Males					
Training	No. crossing[b]	13/13	16/16	14/14	16/16
	Latency (sec)[c]	39.36 ± 35.074	18.04 ± 16.250	25.97 ± 15.244	18.29 ± 11.489
Trial 1	No. crossing	1/13	2/16	3/14	1/16
	Latency (sec.)	112.46 ± 27.202	109.51 ± 30.125	103.87 ± 34.187	113.52 ± 25.933
Trial 2	No. crossing	0/1	0/2	0/3	0/1
	Latency (sec.)	120.00	120.00	120.00	120.00
Short-term memory (1 hr)	No. crossing	0/13	3/16	1/14	2/16
	Latency (sec.)	120.00	107.65 ± 30.525	119.52 ± 1.804	111.63 ± 24.852
Long-term memory (>25 hr)	No. crossing	0/13	1/13	1/13	1/14
	Latency (sec.)[d]	120.00	114.86 ± 18.516	119.45 ± 1.966	111.89 ± 30.337
Females					
Training	No. crossing[b]	15/15	16/16	15/15	15/15
	Latency (sec)[c]	27.40 ± 18.622	32.25 ± 39.117	31.77 ± 63.501	29.27 ± 22.429
Trial 1	No. crossing	1/15	1/16	2/15	1/15
	Latency (sec.)	113.08 ± 26.783	119.35 ± 2.610	118.33 ± 4.433	113.28 ± 26.019
Trial 2	No. crossing	0/1	0/1	0/2	0/1
	Latency (sec.)	120.00	120.00	120.00	120.00
Short-term memory (1 hr)	No. crossing	1/15	2/16	2/15	1/15
	Latency (sec.)	117.69 ± 8.939	116.68 ± 9.211	116.11 ± 10.449	119.38 ± 2.383
Long-term memory (>25 hr)	No. crossing	0/14	0/14	1/13	0/14
	Latency (sec.)[d]	120.00	120.00	114.60 ± 19.459	120.00

[a] Data obtained from Table 18, pp. 139-140, MRID 45630301.
[b] Number of animals crossing to the dark side of the cage.
[c] Animals remaining on the lighted side for 2 minutes were considered to have met criteria.
[d] Excluding animals not reaching criteria at 1 hr memory.

Water Maze: No adverse effects were observed on swimming ability assessed on day 58. No treatment-related differences were observed for the treated groups at any dose level compared to controls with regard to time to escape or number of errors. Data for males and females are summarized in Tables 14 and 15, respectively.

Further analyses of individual animal data showed no apparent effect of methyl parathion treatment on learning and memory under the conditions of the test. The data analyses are presented in Appendix I. As shown in Tables 1 and 2 (Appendix 1), no treatment-related effects between the number of animals with no errors and the number of animals with $\geq$ 10 errors were observed by dose group. As shown in Table 3 (Appendix 1), no treatment-related effects were seen in terms of the number of animals where the second trial was $\leq$ the first trial with regard to time to completion in each of the three trial days for Path B.

TABLE 14. Cincinnati water maze performance - males [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.03	0.30	0.60
		Path B (PND 59, 60, 61)			
Day 1 - Trial 1	Time (sec.)	92.2 ± 42.06	69.6 ± 24.93	90.2 ± 28.01	105.6 ± 61.87
	Errors	12.9 ± 5.51	10.1 ± 5.84	13.7 ± 5.86	14.4 ± 8.26
Day 1 - Trial 2	Time (sec.)	76.5 ± 50.47	101.2 ± 70.75	84.5 ± 50.35	98.8 ± 49.91
	Errors	11.6 ± 9.10	11.0 ± 6.81	11.9 ± 8.71	12.7 ± 7.44
Day 2 - Trial 3	Time (sec.)	68.4 ± 38.61	108.8 ± 64.95	92.5 ± 42.12	89.6 ± 79.35
	Errors	9.5 ± 7.35	10.8 ± 6.88	9.1 ± 6.32	13.2 ± 10.64
Day 2 - Trial 4	Time (sec.)	98.7 ± 62.48	82.1 ± 60.61	123.2 ± 76.50	110.6 ± 57.79
	Errors	9.1 ± 5.42	7.6 ± 5.34	10.5 ± 7.88	12.4 ± 7.24
Day 3 - Trial 5	Time (sec.)	63.3 ± 36.93	40.9 ± 15.15	84.3 ± 40.14	89.1 ± 78.26
	Errors	8.3 ± 7.36	6.8 ± 8.12	8.1 ± 6.12	8.9 ± 7.27
Day 3 - Trial 6	Time (sec.)	46.7 ± 32.66	83.8 ± 74.72	76.4 ± 37.18	67.2 ± 57.74
	Errors	3.6 ± 3.89	6.3 ± 6.28	5.8 ± 4.59	6.3 ± 6.06
		Path A (PND 63, 64, 65)			
Day 1 - Trial 1	Time (sec.)	113.9 ± 78.36	115.5 ± 65.04	125.9 ± 90.31	130.8 ± 57.97
	Errors	16.5 ± 13.43	11.7 ± 9.69	13.1 ± 10.25	18.3 ± 10.32
Day 1 - Trial 2	Time (sec.)	71.8 ± 50.43	78.8 ± 63.78	72.7 ± 44.95	69.7 ± 56.70
	Errors	7.9 ± 8.15	5.4 ± 5.73	5.3 ± 6.97	7.4 ± 9.33
Day 2 - Trial 3	Time (sec.)	83.8 ± 73.34	65.5 ± 58.44	74.0 ± 44.98	76.4 ± 64.36
	Errors	6.9 ± 6.83	5.7 ± 8.32	4.4 ± 4.70	6.0 ± 7.08
Day 2 - Trial 4	Time (sec.)	51.8 ± 38.99	54.4 ± 50.68	48.1 ± 25.83	64.8 ± 52.04
	Errors	3.0 ± 3.55	2.8 ± 3.41	2.3 ± 4.27	3.2 ± 3.59
Day 3 - Trial 5	Time (sec.)	35.1 ± 20.45	34.5 ± 26.00	52.2 ± 39.28	42.3 ± 45.59
	Errors	0.9 ± 1.68	2.0 ± 3.22	2.0 ± 3.21	1.9 ± 3.23
Day 3 - Trial 6	Time (sec.)	37.9 ± 30.50	28.3 ± 15.75	52.4 ± 69.50	49.1 ± 69.46
	Errors	1.4 ± 2.77	0.4 ± 0.81	2.1 ± 5.57	1.2 ± 1.74

[a] Data obtained from Tables 33 & 34, pp. 200-207, MRID 45630301.

TABLE 15. Cincinnati water maze performance - females [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.03	0.30	0.60
Path B (PND 59, 60, 61)					
Day 1 - Trial 1	Time (sec.)	87.8 ± 45.84	84.6 ± 31.70	74.7 ± 30.75	111.1 ± 73.59
	Errors	13.7 ± 8.33	10.8 ± 4.48	11.3 ± 8.09	14.1 ± 8.87
Day 1 - Trial 2	Time (sec.)	126.5 ± 57.91	108.1 ± 68.45	99.4 ± 68.68	90.1 ± 61.45
	Errors	14.8 ± 8.69	13.7 ± 9.45	13.4 ± 7.87	10.3 ± 8.55
Day 2 - Trial 3	Time (sec.)	95.9 ± 72.64	80.8 ± 39.79	92.5 ± 53.21	81.8 ± 62.47
	Errors	11.8 ± 9.66	11.4 ± 9.14	19.1 ± 11.11	12.9 ± 11.30
Day 2 - Trial 4	Time (sec.)	72.6 ± 47.27	98.3 ± 73.11	119.1 ± 56.75	91.7 ± 69.81
	Errors	5.5 ± 4.58	9.0 ± 8.04	10.3 ± 6.40	8.9 ± 7.19
Day 3 - Trial 5	Time (sec.)	61.1 ± 52.54	100.4 ± 65.22	73.8 ± 39.41	66.0 ± 42.83
	Errors	10.1 ± 12.13	11.1 ± 9.56	10.0 ± 8.48	7.1 ± 5.13
Day 3 - Trial 6	Time (sec.)	65.4 ± 46.98	85.3 ± 40.83	81.1 ± 49.51	61.9 ± 32.88
	Errors	5.3 ± 5.67	7.4 ± 5.60	9.9 ± 8.93	5.4 ± 4.70
Path A (PND 63, 64, 65)					
Day 1 - Trial 1	Time (sec.)	104.7 ± 48.27	154.0 ± 76.60	127.5 ± 75.26	135.3 ± 64.98
	Errors	15.3 ± 10.56	20.4 ± 11.30	15.5 ± 12.80	20.8 ± 14.47
Day 1 - Trial 2	Time (sec.)	109.5 ± 73.38	119.6 ± 94.32	72.1 ± 36.93	89.7 ± 62.55
	Errors	7.6 ± 6.96	9.8 ± 9.01	5.9 ± 5.93	8.1 ± 7.81
Day 2 - Trial 3	Time (sec.)	57.9 ± 37.05	86.2 ± 60.24	77.5 ± 49.93	80.7 ± 58.14
	Errors	3.6 ± 4.05	6.8 ± 6.60	7.1 ± 8.22	6.5 ± 7.80
Day 2 - Trial 4	Time (sec.)	43.9 ± 23.99	74.8 ± 77.41	49.5 ± 31.07	51.4 ± 35.02
	Errors	1.7 ± 1.91	3.9 ± 5.86	3.4 ± 6.70	2.2 ± 2.66
Day 3 - Trial 5	Time (sec.)	52.4 ± 41.46	55.9 ± 54.20	61.0 ± 49.23	39.1 ± 27.10
	Errors	1.9 ± 3.18	2.0 ± 4.05	3.1 ± 5.60	1.3 ± 1.98
Day 3 - Trial 6	Time (sec.)	31.8 ± 12.86	41.6 ± 29.06	33.9 ± 15.17	39.5 ± 30.40
	Errors	0.3 ± 0.62	1.1 ± 2.22	1.0 ± 1.52	1.4 ± 2.28

[a] Data obtained from Tables 33 & 34, pp. 200-207, MRID 45630301.

5. Postmortem results:

a. **Brain parameters:** Brain weight and measurement data from perfused animals are given in Table 16. No dose- or treatment-related differences were seen between the treated and control groups for either sex. On day 22, the high-dose males had a significantly ($p \leq 0.05$) longer measurement from anterior to posterior cerebrum compared with the controls. This single difference was considered incidental to treatment because other brain measurements on day 22 were similar to the controls, no effects were found at the other time points, the magnitude of the changes were minimal, there was no dose-response and females were not affected. In addition, brain weights from non-perfused animals on day 70 were similar between the treated and control groups for both males and females.

TABLE 16. Terminal body weight and brain measurement data (mean ± SD) in perfused offspring [a]

Parameter	Dose (mg/kg/day)			
	0	0.03	0.30	0.60
Males				
Day 11				
Terminal body weight (g)	25.90 ± 2.326	25.63 ± 2.450	25.10 ± 2.122	26.30 ± 1.825
Brain weight (g)	1.20 ± 0.068	1.21 ± 0.064	1.21 ± 0.055	1.20 ± 0.039
Brain length (mm)[b]	12.65 ± 0.349	12.59 ± 0.359	12.64 ± 0.264	12.55 ± 0.329
Brain width (mm)[c]	13.69 ± 0.339	13.70 ± 0.165	13.58 ± 0.240	13.61 ± 0.237
Brain length (mm)[d]	16.38 ± 0.457	16.42 ± 0.390	16.41 ± 0.303	16.39 ± 0.338
Brain width (mm)[e]	9.37 ± 0.330	9.31 ± 0.328	9.36 ± 0.412	9.41 ± 0.308
Day 22				
Terminal body weight (g)	61.32 ± 5.674	63.01 ± 5.218	59.68 ± 4.593	61.22 ± 6.195
Brain weight (g)	1.70 ± 0.086	1.75 ± 0.069	1.71 ± 0.076	1.74 ± 0.050
Brain length (mm)[b]	14.20 ± 0.410	14.26 ± 0.274	14.24 ± 0.212	14.51 ± 0.236*
Brain width (mm)[c]	15.04 ± 0.421	15.00 ± 0.465	14.93 ± 0.409	15.01 ± 0.264
Brain length (mm)[d]	18.76 ± 0.469	18.81 ± 0.399	18.69 ± 0.408	18.87 ± 0.337
Brain width (mm)[e]	11.21 ± 0.342	11.27 ± 0.334	11.27 ± 0.401	11.33 ± 0.500
Termination (day 70)				
Terminal body weight (g)	404.5 ± 44.778	385.94 ± 31.861	398.94 ± 29.616	395.59 ± 29.300
Brain weight (g)	2.27 ± 0.109	2.27 ± 0.094	2.27 ± 0.060	2.29 ± 0.104
Brain length (mm)[b]	15.46 ± 0.552	15.59 ± 0.343	15.48 ± 0.422	15.75 ± 0.414
Brain width (mm)[c]	15.55 ± 0.467	15.71 ± 0.476	15.52 ± 0.612	15.68 ± 0.439
Brain length (mm)[d]	21.12 ± 0.535	21.26 ± 0.634	20.96 ± 0.747	21.34 ± 0.687
Brain width (mm)[e]	12.04 ± 0.542	12.27 ± 0.342	12.12 ± 0.431	12.21 ± 0.437
Females				
Day 11				
Terminal body weight (g)	25.55 ± 2.602	25.49 ± 1.962	25.01 ± 1.457	25.43 ± 1.890
Brain weight (g)	1.17 ± 0.073	1.17 ± 0.056	1.15 ± 0.058	1.17 ± 0.048

Brain length (mm)[b]	12.55 ± 0.460	12.47 ± 0.365	12.29 ± 0.342	12.46 ± 0.322
Brain width (mm)[c]	13.53 ± 0.279	13.51 ± 0.268	13.64 ± 0.697	13.50 ± 0.235
Brain length (mm)[d]	16.30 ± 0.473	16.19 ± 0.335	15.91 ± 0.759	16.14 ± 0.446
Brain width (mm)[e]	9.38 ± 0.328	9.44 ± 0.384	9.30 ± 0.328	9.37 ± 0.313
Day 22				
Terminal body weight (g)	59.04 ± 5.190	57.93 ± 3.806	58.83 ± 4.062	57.91 ± 5.835
Brain weight (g)	1.68 ± 0.073	1.69 ± 0.044	1.68 ± 0.053	1.69 ± 0.087
Brain length (mm)[b]	14.17 ± 0.281	14.19 ± 0.230	14.22 ± 0.266	14.23 ± 0.218
Brain width (mm)[c]	14.90 ± 0.464	14.81 ± 0.322	14.85 ± 0.432	14.76 ± 0.579
Brain length (mm)[d]	18.54 ± 0.361	18.48 ± 0.325	18.40 ± 0.284	18.54 ± 0.266
Brain width (mm)[e]	11.17 ± 0.273	11.06 ± 0.314	11.08 ± 0.325	11.14 ± 0.349
Termination (day 70)				
Terminal body weight (g)	233.76 ± 20.175	236.29 ± 20.565	243.73 ± 33.579	246.69 ± 18.104
Brain weight (g)	2.12 ± 0.055	2.11 ± 0.071	2.13 ± 0.072	2.12 ± 0.094
Brain length (mm)[b]	15.41 ± 0.298	15.19 ± 0.474	15.30 ± 0.421	15.31 ± 0.514
Brain width (mm)[c]	15.26 ± 0.361	15.20 ± 0.513	15.28 ± 0.322	15.36 ± 0.474
Brain length (mm)[d]	20.52 ± 0.397	20.53 ± 0.698	20.51 ± 0.499	20.57 ± 0.396
Brain width (mm)[e]	12.05 ± 0.299	11.85 ± 0.447	12.05 ± 0.234	12.10 ± 0.411

aData obtained from Tables 19 & 36, pp. 141-148 & 210-213, MRID 45630301.
[b]Anterior cerebrum to posterior cerebrum. *p ≤ 0.05
[c]Cerebral maximal coronal width.
[d]Anterior cerebrum to posterior cerebellum.
[e]Cerebellar maximal coronal width.
N = 14-16/sex/dose

b. Neuropathology

1) **Macroscopic examination:** No treatment-related effects in the brain or olfactory bulb were reported for male or female offspring at postnatal days 22 or 70. However, offspring at PND 11 were not examined for gross neuropathology.

2) **Microscopic examination:** No significant treatment-related effects were noted on postnatal days 22 or 70. However, microscopic neuropathological examinations were not carried out as per guideline requirement (10/group) on PND 70. No reasons were provided for this. Moreover, it is not clear if the specific neuropathological alterations, outlined in EPA Guideline OPPTS 870.6300, were measured.

3) **Brain Morphometry:** No significant differences were found in cerebellar or cerebral measurements between the control and high-dose animals on days 22 and 70. Data are summarized in Table 17.

Treatment had no adverse effects on the offspring survival, clinical signs, developmental landmarks, body weight, body weight gain, food consumption, motor activity, auditory startle reflex, learning and memory, brain weights, brain morphology, or neuropathology. During the period of direct dosing, 12 pups (5 males and 7 females) from 5 litters at the high dose exhibited tremors post-dosing only on Day 13.

The maternal NOAEL is 0.60 mg/kg/day, the highest dose tested. The maternal LOAEL is not established.

The offspring LOAEL is 0.60 mg/kg/day, based on tremors observed on PND 13. The offspring NOAEL is 0.30 mg/kg/day.

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

C. **STUDY DEFICIENCIES:** None

TABLE 17. Mean (±SD) morphometric data in offspring [a]

Parameter	Dose (mg/kg/day)			
	0	0.60	0	0.60
	Males		Females	
Day 22				
Cerebellum				
Left lobule 8 thickness (μm)	844.04 ± 141.322	876.85 ± 117.102	914.20 ± 109.919	860.05 ± 102.749
Right lobule 8 thickness (μm)	879.84 ± 126.791	869.86 ± 69.191	872.33 ± 101.329	910.47 ± 174.775
Hippocampus				
Line A - right (μm)	2322.16 ± 95.362	2325.51 ± 108.268	2232.20 ± 91.790	2322.11±128.899
Line A - left (μm)	2297.83 ± 73.445	2339.03 ± 124.428	2250.42 ± 87.092	2265.03±157.754
Line B - right (μm)	1461.73 ± 59.479	1496.21 ± 82.752	1532.00 ± 56.972	1479.75±139.524
Line B - left (μm)	1431.53 ± 66.329	1461.33 ± 71.503	1531.63 ± 74.447	1475.20 ±120.761
Corpus callosum - line C (μm)	407.99 ± 29.919	372.86 ± 43.135	385.81 ± 43.129	381.64 ± 46.475
Day 70				
Cerebellum				
Left lobule 8 thickness (μm)	941.33 ± 123.751	942.19 ± 114.783	994.38 ± 119.472	896.32 ± 123.946
Right lobule 8 thickness (μm)	936.80 ± 121.290	922.05 ± 102.535	952.90 ± 78.317	879.73 ± 84.350
Hippocampus				
Line A - right (μm)	2354.07 ± 131.818	2377.57 ± 60.788	2359.27 ± 114.052	2335.90 ± 97.221
Line A - left (μm)	2302.44 ± 138.730	2310.83 ± 63.264	2298.86 ± 119.555	2320.43 ± 74.593
Line B - right (μm)	1796.30 ± 146.589	1764.59 ± 109.639	1688.94 ± 81.924	1714.18 ± 63.916
Line B - left (μm)	1782.20 ± 180.738	1751.03 ± 95.851	1690.70 ± 96.087	1718.41 ± 58.357
Corpus callosum - line C (μm)	420.64 ± 54.373	392.18 ± 27.535	410.94 ± 33.512	409.09 ± 29.054

[a]Data obtained from Tables 24 & 40, pp. 160-161 & 227-228, respectively, MRID 45630301.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The author stated that no maternal toxicity was observed and no functional or neuropathological changes were seen in the offspring at doses that were previously shown to produce significant cholinesterase inhibition. Treatment-related effects were limited to tremors for a small number of pups dosed directly at 0.60 mg/kg/day. The author concluded that at doses previously shown to produce significant cholinesterase inhibition, methyl parathion is not selectively developmentally neurotoxic.

B. **REVIEWER COMMENTS:**

No treatment-related effects were seen in the dams on survival, body weight, body weight gain, food consumption, clinical signs, or reproductive performances.

APPENDIX I

The individual animal data from the learning and memory assessment were analyzed by the reviewer, because it seemed that treated males and females were slower than controls and/or made more errors in trials 3-6 for path B. The methods description (p. 25 of MRID 45630301) suggests that Path A was done first and Path B was done later. In fact, according to the data in the MRID – and as reported in the DER – Path B (PND 59-61) was done before Path A (PND 63-65). Animals were tested twice (10 minutes apart) by measuring how long it took to complete the first path (Path B in a "Y" maze in this case) – learning phase? The two measurements were called trials and there were six total trials done over three days (two trials per day – PND 59-61). Day 2 and 3 can be considered learning/memory and memory phases, respectively. Then the **same animals** did it all over again one day later for three consecutive days (PND 63-65) in the other direction (Path A). It is suggested that Path A data be disregarded for the following reasons: (1) it began only one day after the Path B experiment; (2) the same animals were used; and (3) the animals were required to begin a new path (A), and so there is no continuity between the Path B and A experiments. Furthermore, it is suggested that Day 1 trials (1 and 2) be considered the learning phase and Day 3 trials (5 and 6) be considered the memory phase. [NOTE: Day 2 trials (3 and 4) are essentially a combination of learning and memory and so the two cannot be distinguished in this experiment.]

Analysis of the data. Excel spreadsheets (attached) provide much of the data that will be discussed. Tables 1, 2, and 3 below provide a synthesized version of the data to support the conclusion that there is no apparent effect of methyl parathion treatment on learning and memory under the conditions of the test.

Pairing of the trials: In this type of study in which two trials are run 10 minutes apart and are not averaged, is it proper to "pair" the data? In other words, if each animal is assessed at time zero and then 10 minutes later – and the two values are reported separately and not averaged – isn't the relationship between them important? It seems that the second trial (in terms of errors, failures, and time-to-completion) should always be lower than the first trial. Otherwise, the two trials should be combined. In this analysis, it is assumed that pairing the samples is appropriate.

Number of errors: Using the raw data in the study, Tables 1and 2 below show the number of animals with no errors and the number of animals with $\geq$ 10 errors by dose group. There does not appear to be a relationship between the number of animals with or without errors and methyl parathion dose.

Number of failures: The Excel graphs labeled "Number of failures" (below) shows that for Path B animals the males appear to show an increase in the number of animals that failed across both time and all treated animals (not dose-related), whereas the females did not show as convincing of an effect. However, because many of the failures in both the control and treated groups occurred in the second trial of the day, it is difficult to determine any possible neurobehavioral effect (i.e., the animal did not fail at time zero, but did 10 minutes later).

Time-to-Completion: The data tables in the DER (Tables 14 and 15) show that time-to-completion appears to increase with dose for Path B males and females, especially for Trials 2 and 3 for males and Trial 4 for females. However, it is difficult to evaluate the Trials separately. They need to be examined as pairs (1-2; 3-4; and 5-6). In addition, as stated above, the utility of the Day 2 data is questionable. Table 3 below shows the results in terms of number of animals where the second trial is ≤ the first trial in each of the three trial days for Path B (which one would think would be expected). The results show no real difference by treatment and actually question the veracity of the test given the assumption that the second of paired trials should almost always be less than the first.

Table 1: Number of Animals With No Errors

mg/kg/day	N	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
MALES							
0	15	0	0	0	1	1	2
0.03	16	0	0	0	0	2	1
0.30	15	0	0	0	0	0	3
0.60	15	0	0	0	0	1	1
FEMALES							
0	15	0	0	0	1	0	1
0.03	16	0	0	1	2	1	0
0.30	14	0	0	1	0	0	1
0.60	16	0	0	0	0	1	1

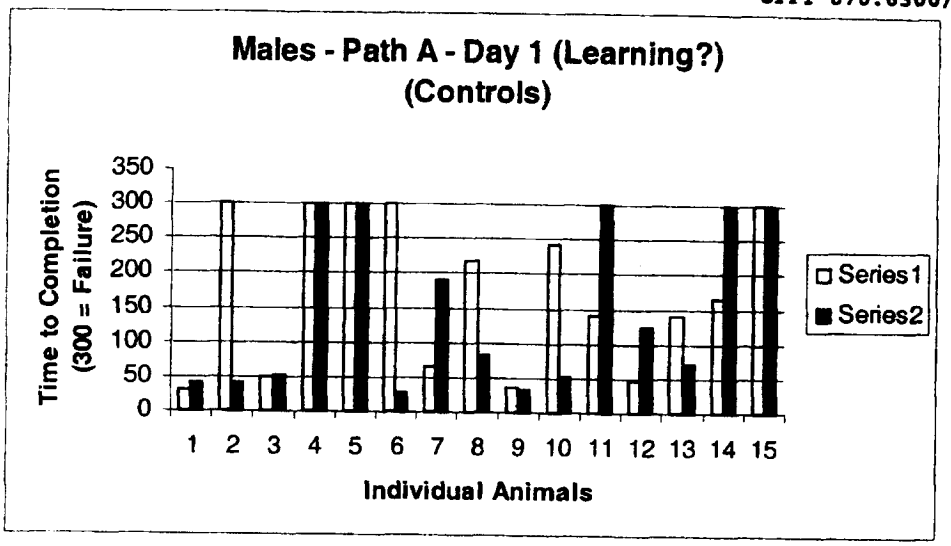
Table 2: Number of Animals With ≥ 10 Errors

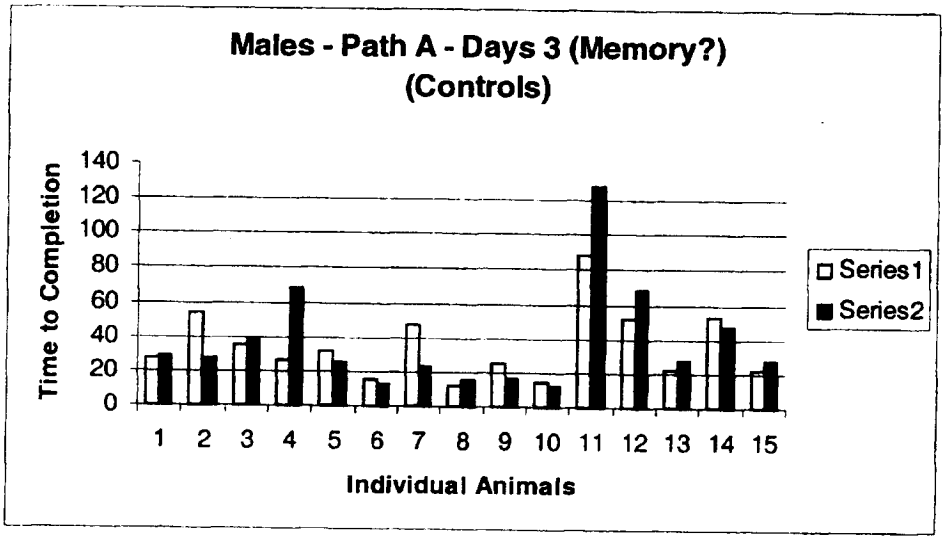
Mg/kg/day	N	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
MALES							
0	15	10	7	5	7	5	1
0.03	16	7	9	9	5	4	4
0.30	15	10	9	7	7	6	3
0.60	15	10	10	7	8	8	2
FEMALES							
0	15	10	9	7	5	5	3
0.03	16	8	10	7	7	7	5
0.30	14	9	9	10	6	6	7
0.60	16	11	6	8	4	4	5

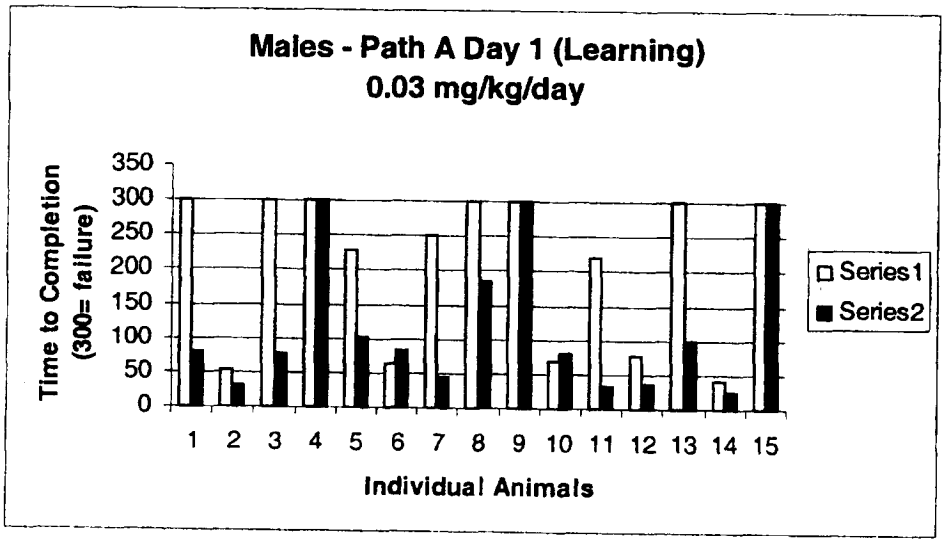
Table 3: Time-to-Completion Comparison of Paired Trials: Number of Animals in Which the Second Trial is ≤ the First Trial (Failures Excluded)

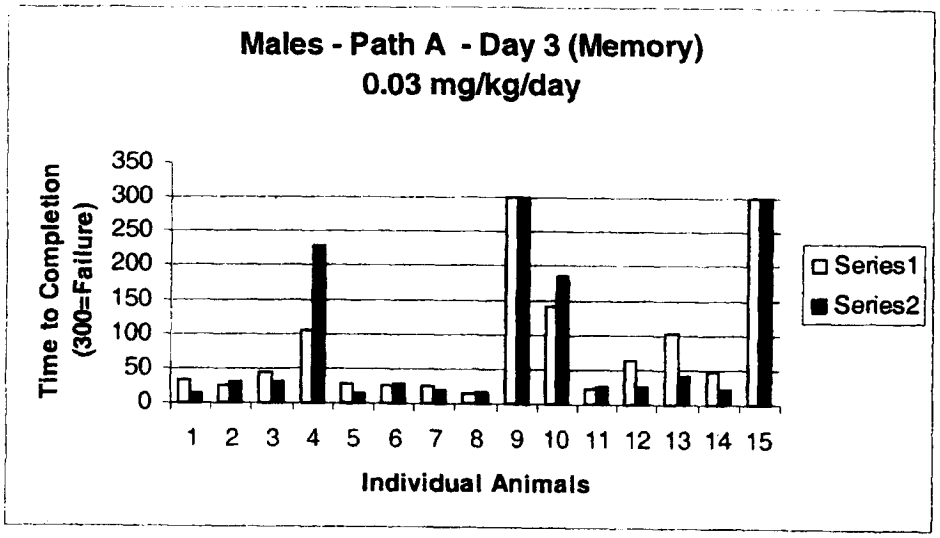
Mg/kg/day	N	Day 1*	Day 2*	Day 3*
MALES				
0	15	9	6	11
0.03	16	5	9	6
0.30	15	6	3	8
0.60	15	6	4	8
FEMALES				
0	15	4	10	9
0.03	16	7	10	9
0.30	14	4	9	7
0.60	16	9	6	9

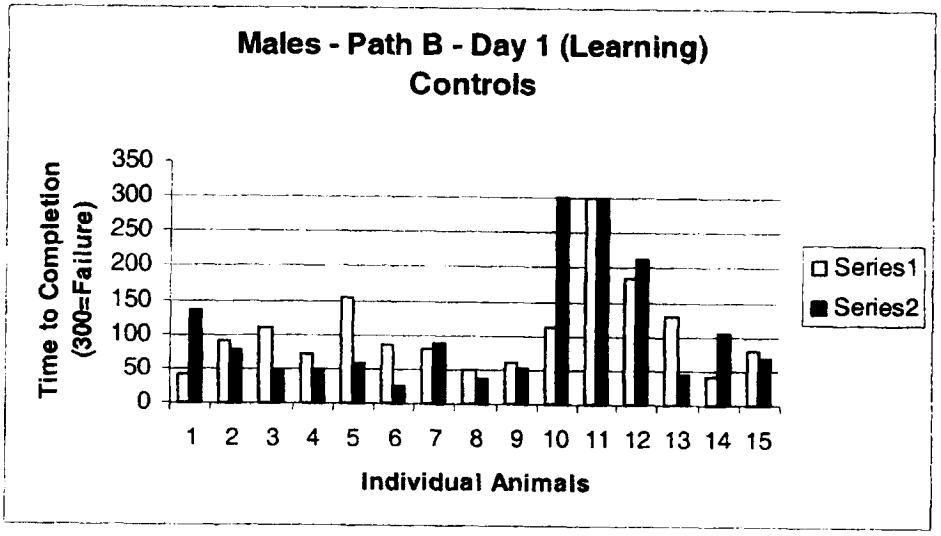
* Day 1 (Trial 1 is the first trial and Trial 2 is the second); Day 2 (Trial 3 is the first trial and Trial 4 is the second); and Day 3 (Trial 5 is the first trial and Trial 6 is the second).

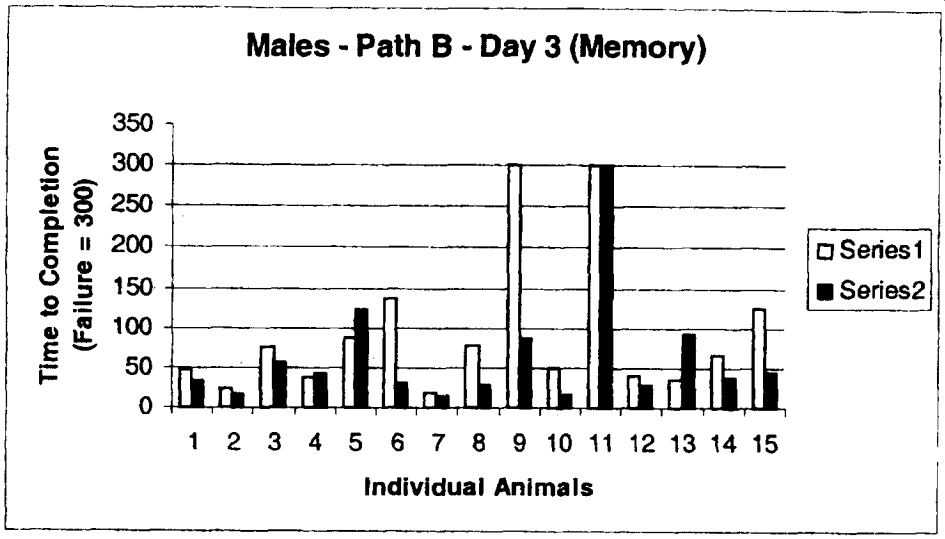

Males - Path A - Day 1 (Learning?)
(Controls)
Time to Completion
(300 = Failure)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

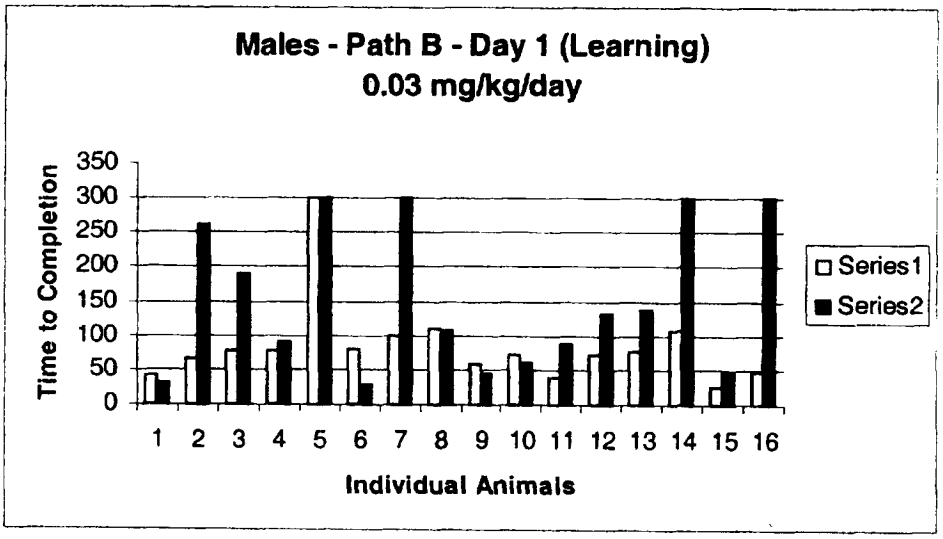

Males - Path A - Days 3 (Memory?)
(Controls)
Time to Completion
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

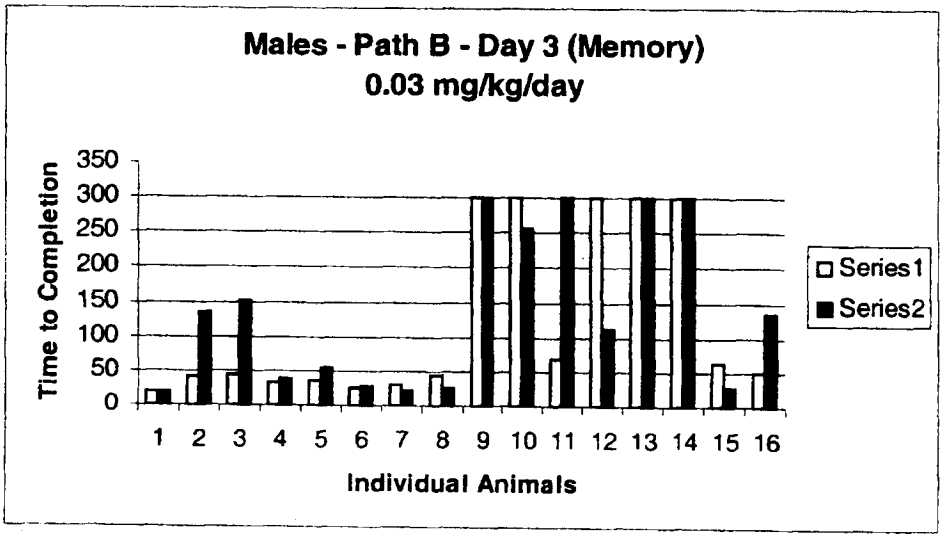

Males - Path A Day 1 (Learning)
0.03 mg/kg/day
Time to Completion
(300= failure)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

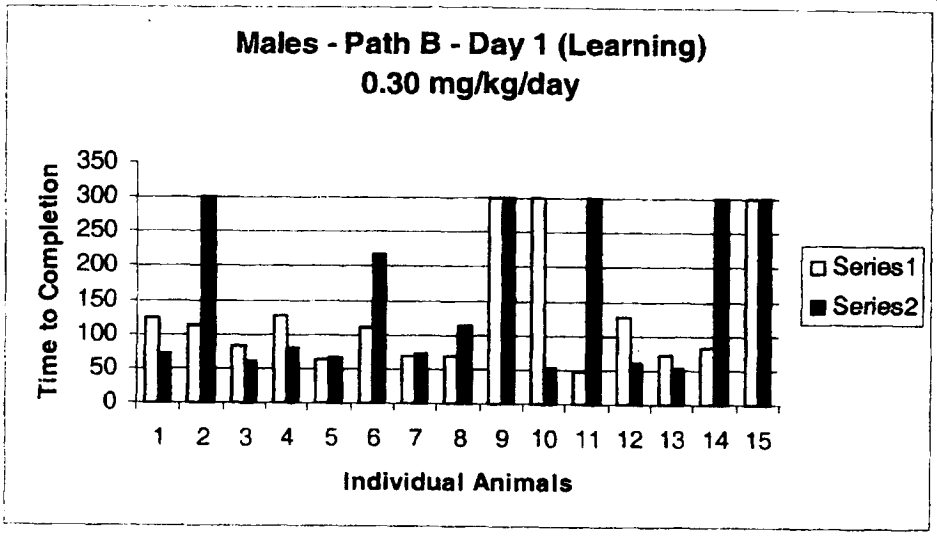

Males - Path A - Day 3 (Memory)
0.03 mg/kg/day
Time to Completion (300=Failure)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

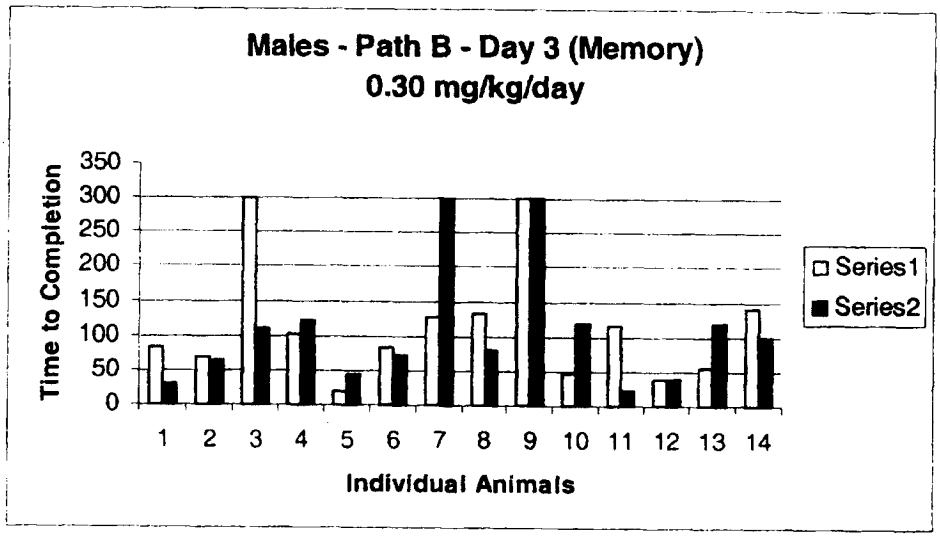

Males - Path B - Day 1 (Learning)
Controls
Time to Completion (300=Failure)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

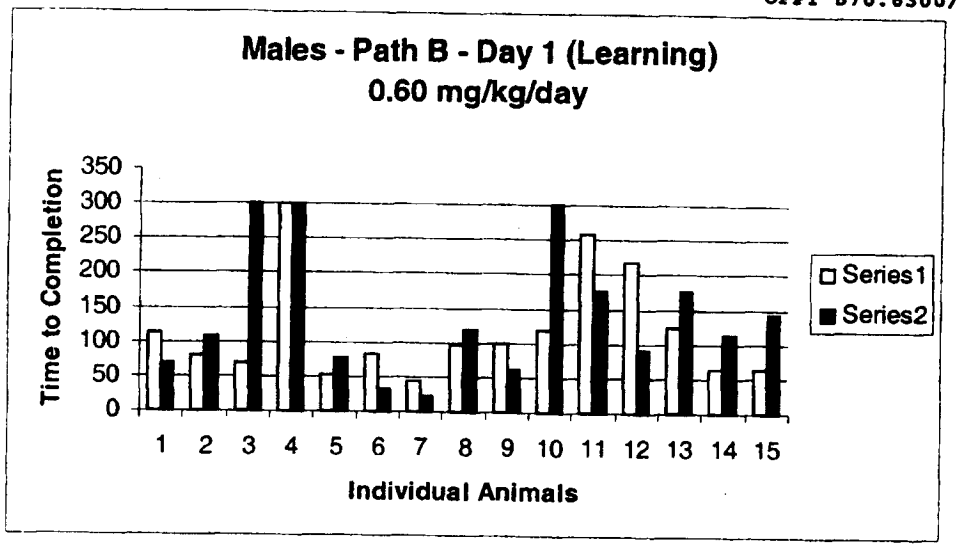

Males - Path B - Day 3 (Memory)
Time to Completion (Failure = 300)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

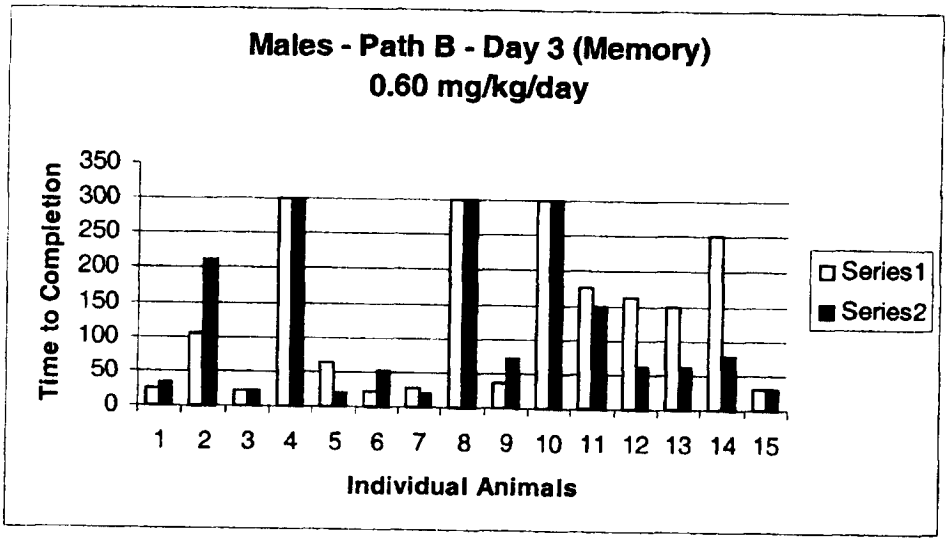

Males - Path B - Day 1 (Learning)
0.03 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Individual Animals
Series1
Series2

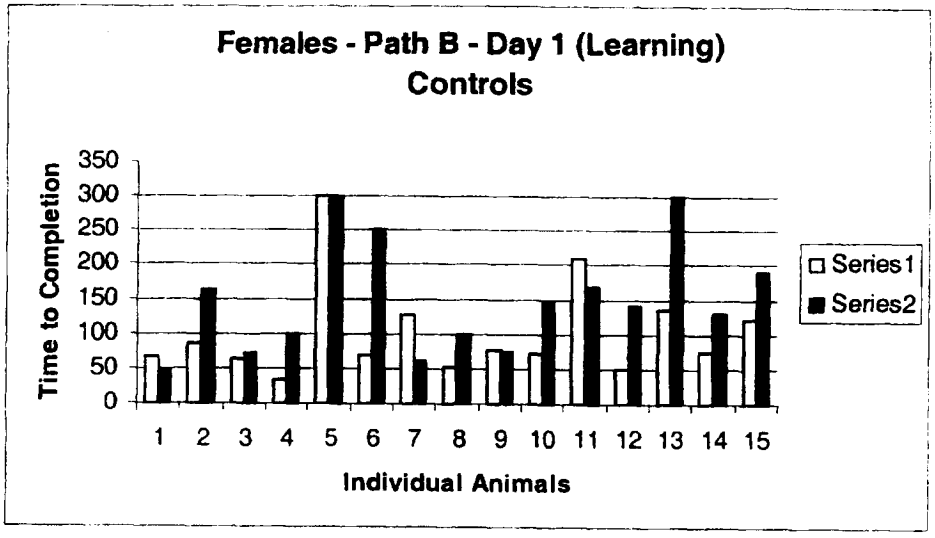

Males - Path B - Day 3 (Memory)
0.03 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Individual Animals
Series1
Series2

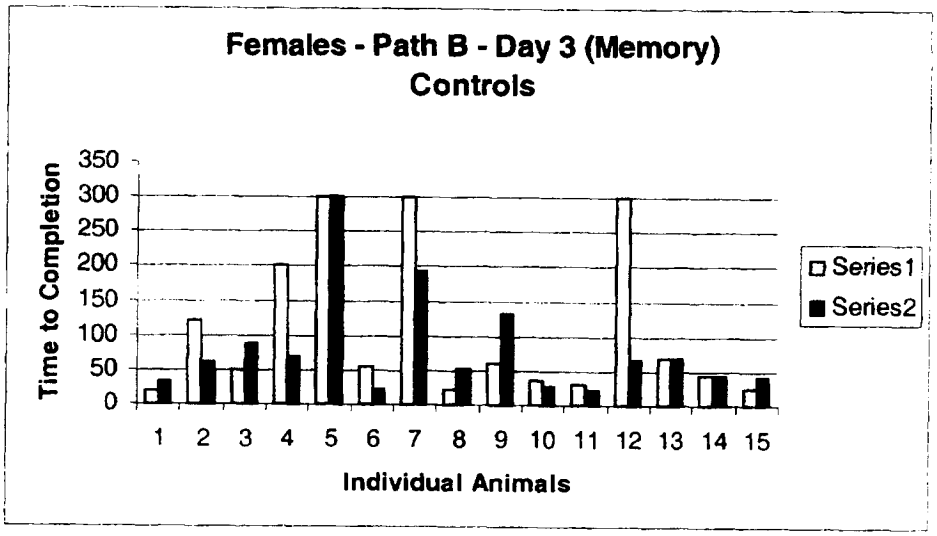

Males - Path B - Day 1 (Learning)
0.30 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

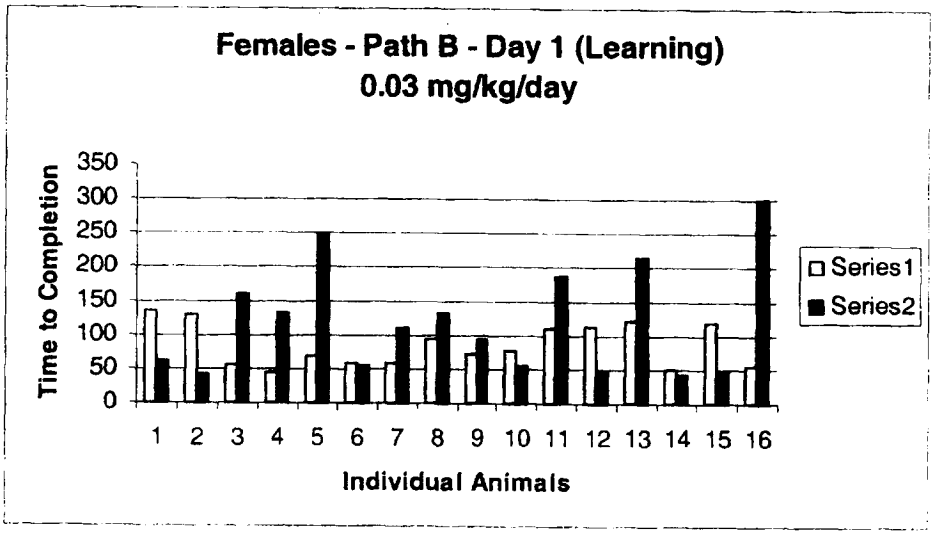

Males - Path B - Day 3 (Memory)
0.30 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Individual Animals
Series1
Series2

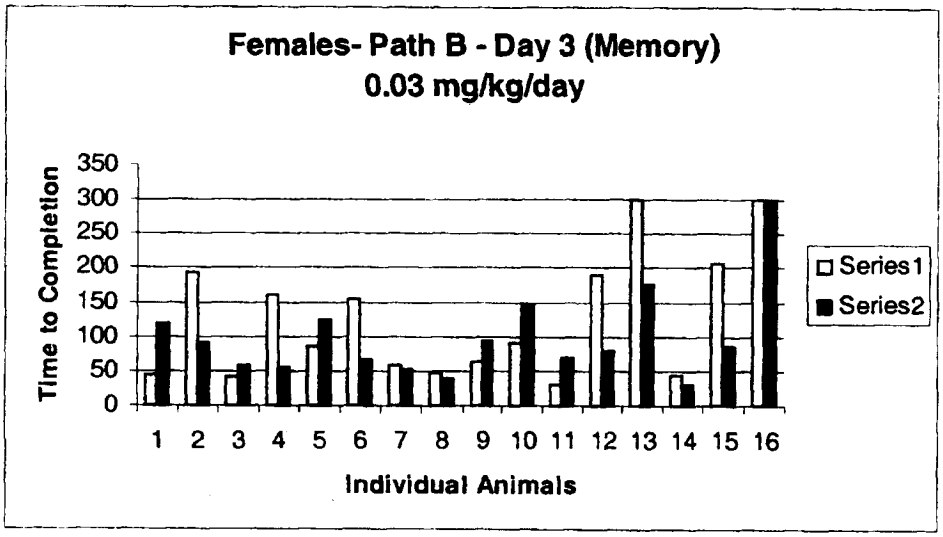

Males - Path B - Day 1 (Learning)
0.60 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

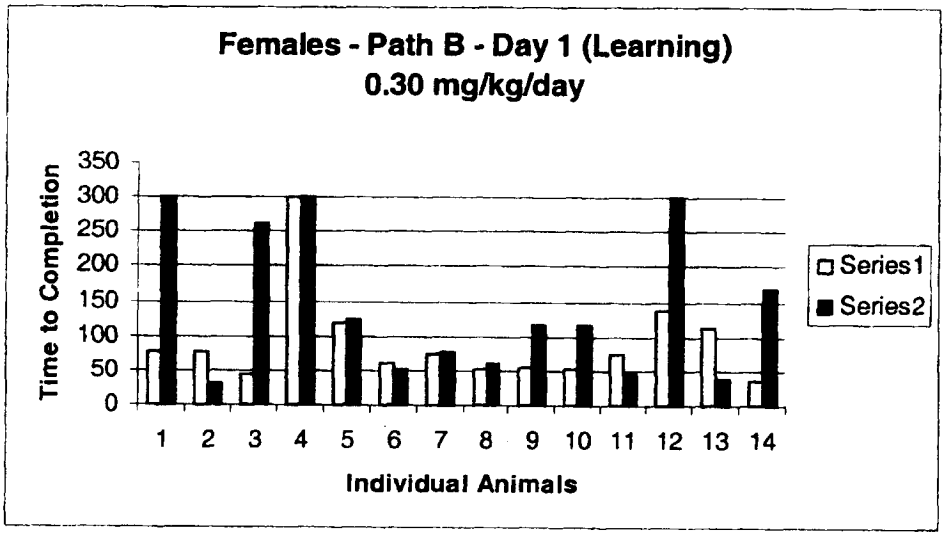

Males - Path B - Day 3 (Memory)
0.60 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

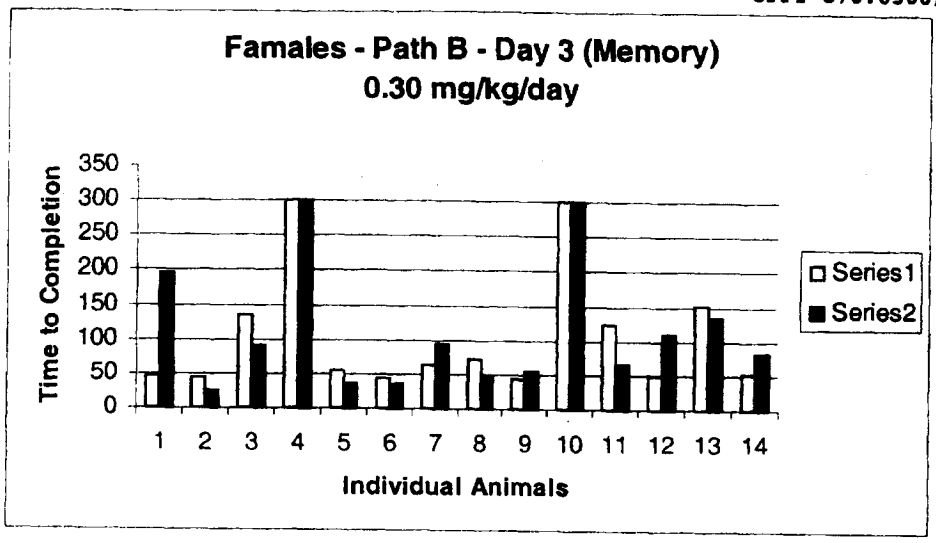

Females - Path B - Day 1 (Learning)
Controls
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

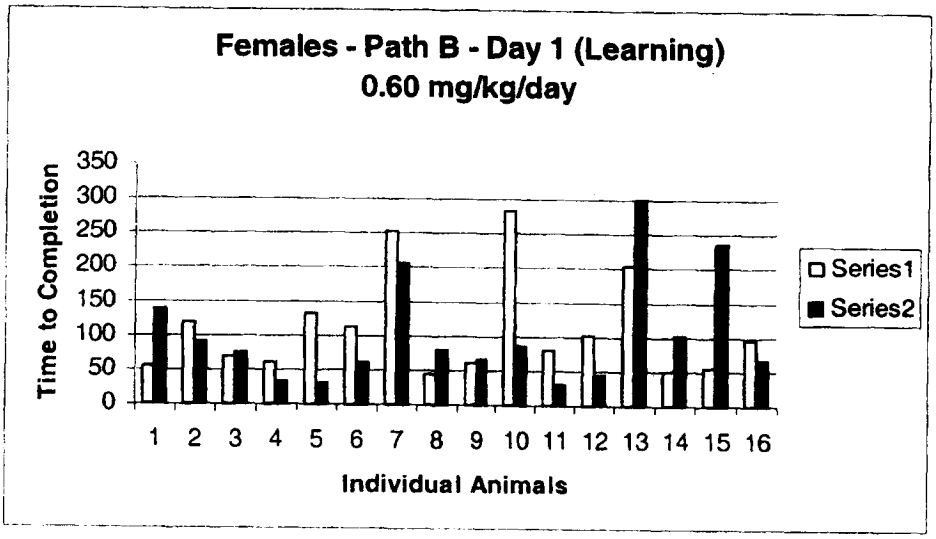

Females - Path B - Day 3 (Memory)
Controls
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Individual Animals
Series1
Series2

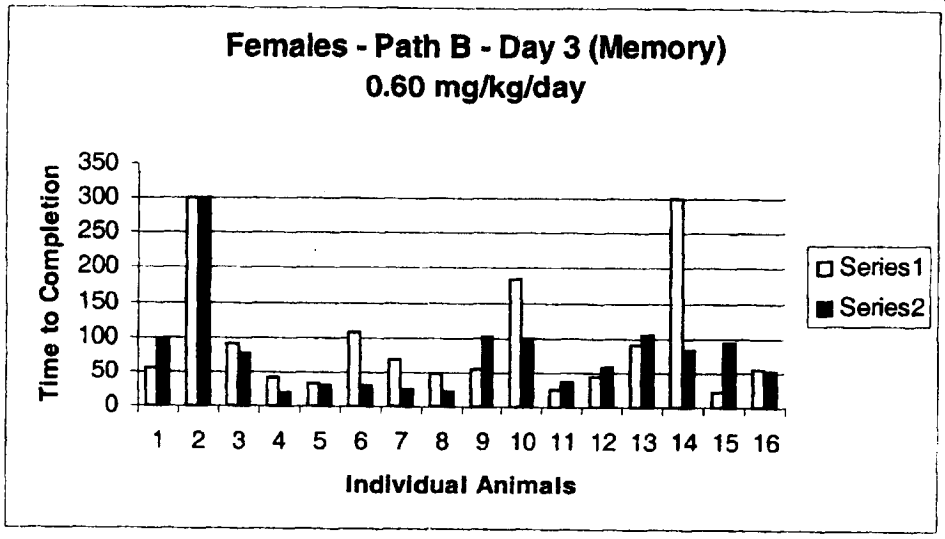

Females - Path B - Day 1 (Learning)
0.03 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Individual Animals
Series1
Series2

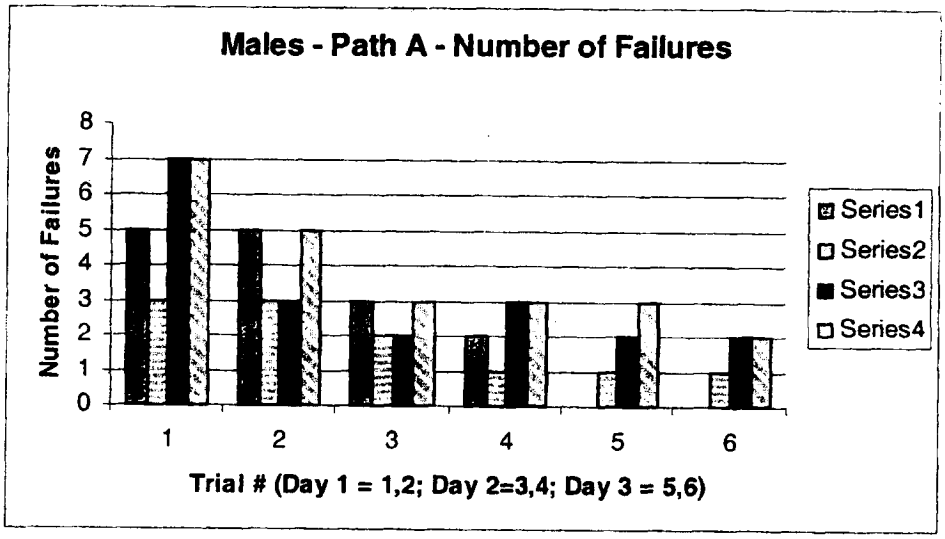

Females- Path B - Day 3 (Memory)
0.03 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Individual Animals
Series1
Series2

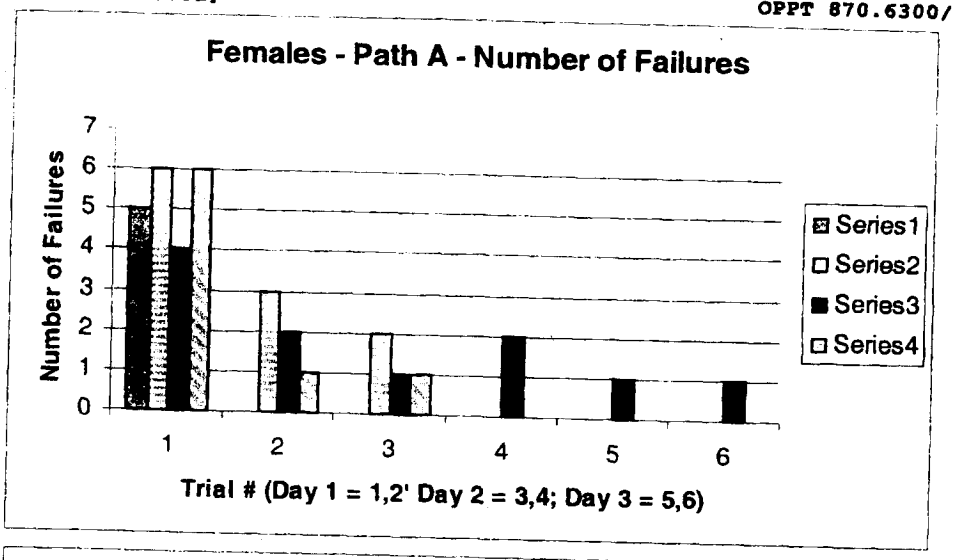

Females - Path B - Day 1 (Learning)
0.30 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Individual Animals
Series1
Series2

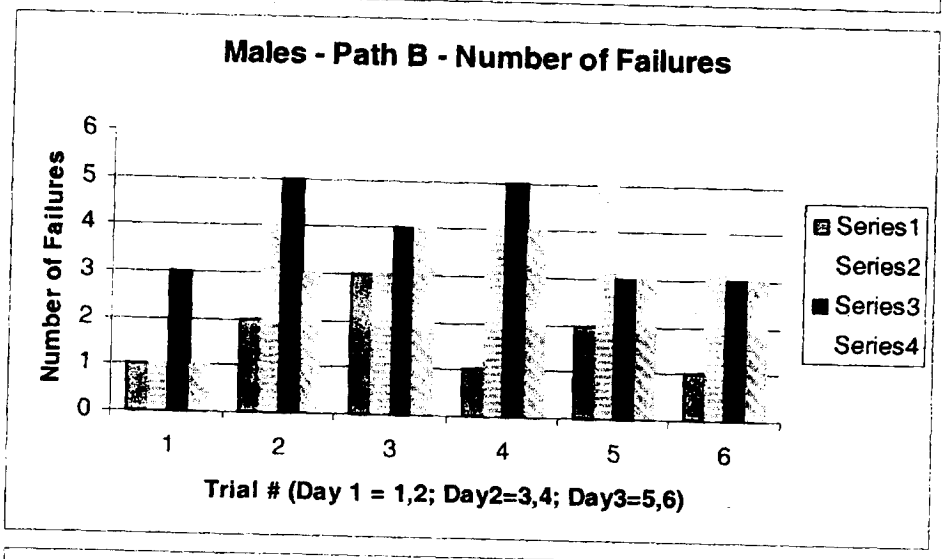

Famales - Path B - Day 3 (Memory)
0.30 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Individual Animals
Series1
Series2

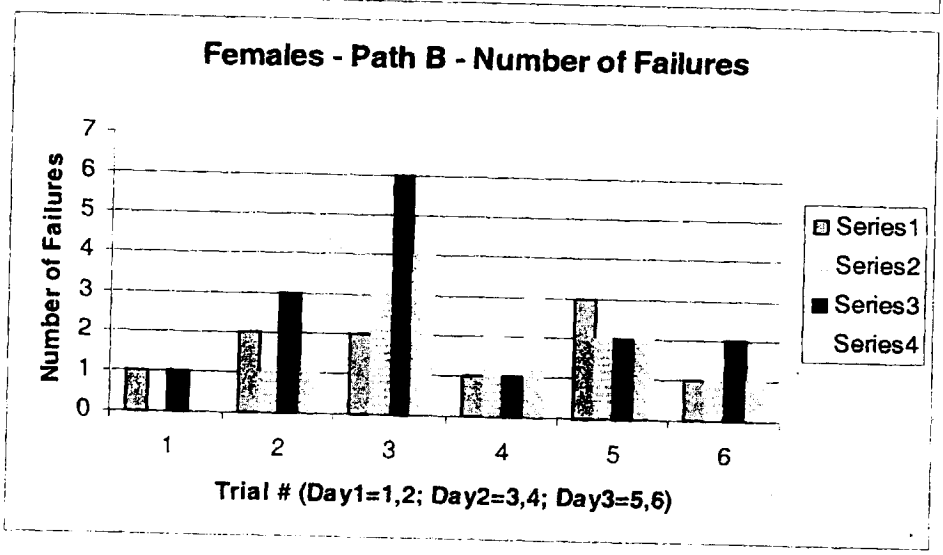

Females - Path B - Day 1 (Learning)
0.60 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Individual Animals
Series1
Series2


Females - Path B - Day 3 (Memory)
0.60 mg/kg/day
Time to Completion
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Individual Animals
Series1
Series2


Males - Path A - Number of Failures
Number of Failures
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6
Trial # (Day 1 = 1,2; Day 2=3,4; Day 3 = 5,6)
Series1
Series2
Series3
Series4


Females - Path A - Number of Failures
Number of Failures
0
1
2
3
4
5
6
7
1
2
3
4
5
6
Trial # (Day 1 = 1,2' Day 2 = 3,4; Day 3 = 5,6)
Series1
Series2
Series3
Series4


Males - Path B - Number of Failures
Number of Failures
0
1
2
3
4
5
6
1
2
3
4
5
6
Trial # (Day 1 = 1,2; Day2=3,4; Day3=5,6)
Series1
Series2
Series3
Series4


Females - Path B - Number of Failures
Number of Failures
0
1
2
3
4
5
6
7
1
2
3
4
5
6
Trial # (Day1=1,2; Day2=3,4; Day3=5,6)
Series1
Series2
Series3
Series4