DATA EVALUATION RECORD

DIMETHOATE/035001

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 45529703

Prepared for

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

Prepared by

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Task No. 02-01

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DATA EVALUATION RECORD
TXR#: 0050139

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

PC CODE: 035001 **DP BARCODE:** D278940
SUBMISSION NO.: S605760

TEST MATERIAL (PURITY): Dimethoate (99.1%w/w)

SYNONYMS: Phosphorodithioic acid, 0,0-dimethyl S-[2-methylamino)-2-oxoethyl]ester

CITATION: Meyers, D. P. (2001) Dimethoate. Developmental neurotoxicity study in the CD rat by oral gavage administration. Huntington Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Laboratory report number CHV/069; 003881, October 19, 2001. MRID 45529703. Unpublished

SPONSOR: Cheminova A/S (EPA Company No. 4787), P.O. Box 9, DK-7620 Lemvig, Denmark.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45529703), Dimethoate (99.1% a.i., batch # 20522-00) was administered to 24 parent female Crl:CD®BR rats per dose by gavage at dose levels of 0, 0.1, 0.5, or 3.0 mg/kg bw/day from gestation day 6 through postnatal day 10, and to the offspring from postnatal day 11 to postnatal day 21 inclusive. A Functional Operational Battery was performed on 10 dams/dose on gestation days 12 and 18 and lactation days 4 and 10. Offspring were evaluated as follows: age-appropriate functional observation battery on days 4, 11, 21, 35, 45, and 60, automated motor activity on days 13, 17, 22, and 60; assessment of auditory startle response days 23/24 and 60/61, assessment of learning and memory (Morris Water Maze) at postnatal days 23/24, and at postnatal day 61/62 (separate groups), brain weights on days 11, 21, and 65, and brain histopathology and morphometrics on days 21 and 65. Pup physical development was assessed by bodyweight, and sexual maturation of females was assessed by age at vaginal opening. Maturation of males was assessed by age at completion of balano-preputial separation.

There were no treatment-related effects, for maternal animals. **The maternal LOAEL for Dimethoate in rats is not identified. The maternal NOAEL is 3 mg/kg/day.**

For offspring, there were no effects on body weight, food consumption, clinical signs, auditory startle parameters, learning and memory evaluations, brain weight, or histopathological evaluations at any time point. There was no effect on litter size or pup weight at birth, but there was an increase in pup death during early lactation. The number of pup deaths was similar in control and low dose groups (15 deaths in 10 litters for controls, 11 deaths in 6 litters at 0.1 mg/kg/day), but was increased at 0.5 mg/kg/day (43 deaths in 10 litters, including 1 total litter loss) and at 3.0 mg/kg/day (89 deaths in 14 litters, including 3 total litter losses). There were also decreased activity levels, as measured in the FOB, at 3.0 mg/kg/day, and changes in automated motor activity measures (decreased rearing in females at 3.0 mg/kg/day on PND17; dose-related increases in horizontal activity in males at 0.5 mg/kg/day [65%] and 3.0 mg/kg/day [122%] on PND17). **The offspring LOAEL is 0.5 mg/kg/day, based on increased pup death and increases in motor activity. The offspring NOAEL is 0.1 mg/kg/day.**

A comparative cholinesterase study (MRID 45529702), using the same doses and dose schedule as the current study, found significant inhibition of plasma, RBC, and brain cholinesterase in dams and fetuses on GD20 after 3 mg/kg/day dimethoate, and significant decreases in brain cholinesterase inhibition (10%) in dams and fetuses given 0.5 mg/kg/day. Pups showed the same pattern of effects on PND21. Based on that study, the NOAEL for cholinesterase inhibition following repeated dimethoate exposure is 0.1 mg/kg/day based on brain cholinesterase in adults and offspring.

This study is classified Acceptable/Guideline and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6).

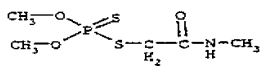
COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided. In addition, a GLP Compliance Review and Data Audit of the study was conducted by EPA in February of 2001 after completion of the in-life portion of the study (MRID 45609601). No adverse GLP findings were noted with respect either to GLP compliance or the study data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Dimethoate

Description:	white solid
Lot/Batch #:	205-22-00
Purity:	99.1 % a.i.
Compound Stability:	5 years
CAS # of TGAI:	60-51-5



2. Vehicle and/or positive control: reverse osmosis water

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD®BR	
Age at study initiation:	10-11 wks	
Wt. at study initiation:	219-315 g	
Source:	Charles River UK Limited, Margate, Kent, England	
Housing:	Individually or with litter in stainless steel grid or solid polypropylene cages	
Diet:	UAR VRF1 pelleted rodent diet (Usine d'Alimentation Rationale, France), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	Up to 15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 5 days	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: October 16, 2000; End: January 22, 2001

2. Study schedule: The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day 6 through postnatal day 10. Pups were weaned on postnatal day 21, after which time maternal animals were killed. F1 pups remained on study until postnatal days 63-67 (study termination).

3. Mating procedure: Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. After successful mating, each pregnant female was placed into an individual cage with a solid bottom and bedding, where the dam was maintained through gestation and lactation.

4. Animal assignment: Mated females were assigned to group and cage position in sequence, so that animals mated on any one day were evenly distributed among treatment groups. The allocation of mated females was adjusted so that more than one female from a given litter was not allocated to the same dose group. Dose groups are 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). One pup/sex/litter was allocated on postnatal day 4 to each of the following: motor activity, auditory startle response habituation and auditory startle pre-pulse inhibition, learning and memory at postnatal day 23/24, learning and memory at postnatal day 60, and sacrifice and brain examination on postnatal day 11. The allocation of one pup/sex/litter for

each test was followed for control, low- and mid-dose groups. However, due to increased pup mortality at the high-dose, it was necessary to allocate some pups from two litters (litters 62 and 72) to two behavioral tests.

TABLE 1. Study Design

Experimental Parameter	Dose (mg/kg/day)			
	0	0.1	0.5	3
Maternal Animals				
No. of maternal animals assigned	24	24	24	24
FOB (GD 12, 18; LD 4, 10)	10	10	10	10
Offspring				
Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PND 13, 17, 22, 59)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 23/24, 60/61)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PND 23/24, 61/62)	10/sex	10/sex	10/sex	10/sex
Brain weight				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 21	10/sex	10/sex	10/sex	10/sex
PND 63-67	10/sex	10/sex	10/sex	10/sex
Neuropathology				
PND 11	10/sex	10/sex	10/sex	10/sex
PND 21	10/sex	10/sex	10/sex	10/sex
PND 63-67	10/sex	10/sex	10/sex	10/sex

5. **Dose selection rationale:** Dose levels were chosen based on the results from oral gavage dose range-finding (Report CHV068/00129; MRID 45529701) and cholinesterase (Report CHV070/012226; MRID 45529702) studies in CD rats. Results from these studies are presented in separate DERs.

6. **Dosage administration:** All doses were administered once daily to maternal animals by gavage, on gestation day 6 through postnatal day 10, 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 gestation day 17; the dosage volume then remained constant to postnatal day 1. From postnatal day 1, dosing volumes were once again calculated based on the most recent body weight. Offspring were dosed by gavage in a volume of 5mL/kg based on the most recent body weight from postnatal day 11 to postnatal day 21 inclusive, except that animals scheduled for terminal sacrifice on postnatal days 11 or 21 were not dosed on the day of sacrifice. Controls received reverse osmosis water (vehicle) only. The dosage level for each group was not known by the observer in the animal unit, and those involved in the necropsy, histology, or pathology.

7. **Dosage preparation and analysis:** Formulations were prepared weekly. The highest required concentration (0.6 mg/mL) was prepared by mixing an appropriate amount of test substance with reverse osmosis water and mixing with a magnetic stirrer. The lower

concentrations (0.02 and 0.01 mg/mL) were then prepared by serial dilution. Dosing solutions were refrigerated for storage. Prior to the start of the study, stability of the test substance in water was evaluated for a period of 2 days at room temperature and 15 days refrigerated. Homogeneity (top, middle, and bottom) was not evaluated. Single samples were taken from each dosing solution prepared for use during the first week of treatment and during the first week of lactation; duplicate HPLC assays of each dosing solution were performed for concentration analysis.

Results:

Homogeneity Analysis: not performed.

Stability analysis: The mean concentrations of dosing solutions remained within 4% of nominal after periods of 2 days at room temperature or 15 days refrigerated.

Concentration Analysis: The mean analytical concentrations of test solutions were within 1.3% of nominal. The precision of duplicate analyses was #0.5%.

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 and daily cage-side observations were conducted for maternal animals. Gross observations of the dams were conducted daily as follows: prior to treatment, as each animal was returned to the cage, at the end of dosing for each group, between 1 and 2 hours after completion of dosing, and as late as possible during the work day.

Ten dams per group were observed outside the home cage at least twice during the gestation dosing period (days 12 and 18) and twice during the lactation dosing period (days 4 and 10) prior to dosing. The following functional observations were recorded.

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 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.

Observers were unaware of the treatment group of the subjects. Subjects were also scored for ease of removal from the cage and reactivity to handling. Observations of gait, grooming, palpebral closure, posture, activity counts, rearing counts, tremors, twitches, convulsions, urination, and defecation were made for one minute in an Open field (650 x 500 mm) divided into six sectors.

Individual maternal body weight data were recorded on gestation days 0, 3, 6, 10, 14, 17, and 20. During lactation, dams were weighed on postnatal days 1, 4, 7, 11, 14, 17, and 21.

Food consumption was recorded on gestation days 0-2, 3-5, 6-9, 10-13, 14-16, and 17-19 and on postnatal days 1-3, 4-6, 7-10, 11-13, 14-16, and 17-20.

From gestation day 20, dams were checked 3 times/day for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Approximate numbers of live and dead offspring were recorded during parturition.

b. Offspring:

1. **Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day) 0. Live pups were counted, sexed and weighed individually for each litter on postnatal days 1, 4, 7, 11, 14, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. Additional observations were made on days of direct dosing to pups (PNDs 11-21): pre-dosing, as the animal was returned to the home cage, at the end of dosing for each group, and as late as possible in the working day.

   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 38, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 28, female offspring were examined daily for vaginal patency. The age of onset was recorded.

3. **Postweaning observations:** After weaning on postnatal day 21, offspring were examined twice daily for mortality or morbidity. A full physical exam was performed weekly, up to study termination. Individual offspring body weight data were recorded weekly from postnatal day 28 until termination at postnatal day 63-67.

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

5. **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, a total of 10 offspring/sex/group (one male or one female from each litter) was examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed.

   Postnatal day 4: A clear arena with a floor size of 30 x 20 cm and side walls of 4.5 cm was utilized. An FOB activity sheet (paper sheet marked with concentric circles) was placed underneath the arena. The animal was then placed in the center of the FOB activity sheet and observed over a one-minute recording period. The following parameters were assessed: righting reflex, number of sections entered, maximum distance traveled, maximum pivoting angle, physical condition (skin color, physical abnormalities, cold-to-touch) locomotor incoordination, tremors, convulsions, and excessive backward movement. The arena was disinfected between each use to prevent activity from being influenced by olfactory cues from previous rats.

   Postnatal day 11: The same arena was used as was used for postnatal day 4; however, the paper placed below the arena was divided into 9 equal segments. The animal was then placed in the center of the FOB activity sheet and observed over a one-minute recording period. The following parameters were assessed: righting reflex, number of sections entered, number of rearings, grooming, urination, physical condition (skin color, physical abnormalities, cold-to-touch) locomotor incoordination, tremors, convulsions, and excessive backward movement. The arena was disinfected between each use to prevent activity from being influenced by olfactory cues from previous rats.

   Postnatal days 21, 35, 45, and 60: The following observations were graded and recorded. (The observations on postnatal day 21 were made after completion of counting, sexing, and weighing, and before the parent was removed for necropsy).

In the Hand Observations	Standard Arena Observations
Removal from cage Salivation Lacrimation Piloerection Exophthalmus Reactivity to handling Pupil Closure reflex (Day 35 only)	Palpebral closure Posture Gait Tremor Twitch Convulsion Activity Rearing Grooming Urination Feces

6. **Motor activity testing:** Motor activity was evaluated in 10 rats/sex/dose on days 13, 17, 22, and 59. Animals were placed in plastic cages and were continuously monitored over a 1-hour period. (On day 13, it was recorded if eyes were open or closed, and on days 13 and 17, motor activity was monitored before dosing). An automated activity monitoring system

collected data over successive 6-minute intervals by recording infra-red light source break frequency within the cage. Low beam detectors were set 3.5 cm above the cage floor to monitor ambulatory activity, while high-beam detectors monitored rearing activity. Due to small animal size on postnatal days 13 and 17, a raised insert was placed in the cage so that activity would be monitored.

7. **Auditory startle reflex habituation and pre-impulse inhibition of startle:** Auditory startle reflex habituation and pre-impulse inhibition of startle testing was performed on 10 offspring/sex/dose on postnatal days 22/23 and 60/61 using an automated system.

   Animals were acclimated for 5 minutes to background noise. Mean startle amplitudes were recorded for 5 consecutive blocks of 10 trials for the auditory startle habituation testing. The startle stimulus consisted of 40-millisecond bursts of white noise at 90% intensity (105 dB) against a background noise level of 70 dB, with inter-stimulus interval of 12 seconds.

   For the pre-impulse inhibition of startle testing, mean startle amplitudes were recorded for 10 trials with a pre-pulse sound immediately preceding the startle stimulus and for 10 trials without a pre-pulse. The startle stimulus consisted of 50-millisecond bursts of white noise at 100% intensity (118 dB) and the pre-pulse consisted of a 50-millisecond pulse of white noise at 70% intensity (85 dB) preceding the startle stimulus by 50 milliseconds. A total of 20 trials (10 each) were presented in a pseudo-random order with inter-trial intervals of 10, 12, 14, or 16 seconds.

8. **Learning and memory testing:** Learning and memory testing was performed in 10 offspring/sex/dose on postnatal days 23/24 and 61/62 using a Morris water maze (a separate group was evaluated at each time point). A series of 3 trials was conducted on each of 4 consecutive days. The water maze consisted of a circular white plastic pool (90 cm diameter; 30 cm deep at days 23/24 and 140 cm diameter, 45 cm deep at days 61/62). The maze was filled with water at 29±3ºC and made opaque with a nontoxic opacifier (Opacifer 621). A 6 cm square platform was concealed at a fixed position 1.5 cm below the surface of the water. Three starting points were identified at the edge of the pool. Each animal received 3 consecutive trials on each day of testing. For the first trial, the animal was placed on the escape platform for 30 seconds prior to testing. The animal was then placed into the water at the edge of the pool and given a maximum of 90 seconds to swim to the platform. A different starting point was used for each trial. The time to reach the platform and the number of quadrants crossed were recorded. The rat was allowed to remain on the platform for 30 seconds after each trial. If the rat did not find the platform within 90 seconds, it was placed on the platform for 30 seconds and a latency of 90 seconds was recorded.

9. **Postmortem observations:**

a. **Maternal animals:** Maternal animals were sacrificed by carbon dioxide inhalation on postnatal day 21. Females whose litter died during lactation were sacrificed on the day the last offspring died. Adult females were subjected to a detailed macroscopic necropsy, and the number of implantation sites was recorded. Specimens of abnormal tissues were retained in fixative, and mammary tissue was retained from females whose litters died early in

lactation.

b. Offspring:

Animals not selected for neuropathologic evaluation: Pups culled on PND 4 or killed before PND 21 were sacrificed by i.p. injection of sodium pentobarbitone or by carbon dioxide inhalation. Offspring killed on day 11 were sacrificed by intraperitoneal injection of barbiturate. Offspring killed on day 21 or at study termination, but not selected for neuropathological evaluation, were sacrificed by carbon dioxide inhalation.

Sporadic neonatal deaths, and offspring culled on PND 4, were not necropsied. Weanling offspring and other offspring dying during or following the late lactation period were subject to detailed macroscopic necropsy, and specimens of abnormal tissue were retained in fixative. In addition, for offspring killed on day 21 or at study termination but not selected for neuropathological examination, brains were removed, weighed, and fixed in 10% neutral buffered formalin (but not examined histopathologically).

Animals selected for neuropathologic evaluation: The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 11, 21 or 63-67. These animals were subjected to postmortem examinations as described below.

At postnatal day 11, ten pups/sex/group were selected for brain weight measurements. The brain was removed and fixed for 24 hours by immersion in 10% neutral buffered formalin after opening the calvarium. Brain weights were recorded after fixation and removal of the brain from the skull. The brains from all pups of all groups were embedded in paraffin, sectioned at 4-5 ：m, and stained with hematoxylin and eosin. Sections included coronal sections (olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, cerebrum, tectum, tegmentum, medulla oblongata) and mid-sagittal sections (cerebellum, pons). The sections were not examined microscopically.

At postnatal day 21, up to ten pups/sex/group were sacrificed by intraperitoneal injection of barbiturate and perfused with gluteraldehyde and paraformaldehyde, followed by immersion in glutaraldehyde and paraformaldehyde. The brain was transected from the spinal cord above the first cervical spinal nerve. The brain length was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum. The width was measured at the widest part of the cerebral hemispheres, and the brain was weighed.

Tissues listed below from all dose groups were embedded in paraffin and were sectioned for control and high-dose animals. Tissues were sectioned at 4-5 ：m and stained with hematoxylin and eosin. Only brains were sectioned for mid- and low-dose animals. Histopathological evaluations included the tissues listed below; only control and high-dose animals were examined microscopically.

BRAIN: **Coronal sections** (olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, tectum, tegmentum, medulla oblongata) and **mid-sagittal sections** (cerebellum, pons) were evaluated qualitatively.

The following brain morphometric measurements were performed:

Thickness of the neocortex (distance from the pial surface to the top of the white matter was measured along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits the greatest thickness)

Corpus callosum (thickness at the midline)

Hippocampus (greatest dorsal-ventral thickness)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between the tip and base)

External germinal layer.

TISSUES OTHER THAN BRAIN: eye, thyroid/parathyroid, thymus, lungs, heart, liver, kidneys, adrenals, pancreas, spleen, GI tract (stomach, duodenum, jejenum, ileum, cecum, colon, rectum), ovaries, uterus, testes, epididymis, prostate, pituitary, mandibular and mesenteric lymph nodes, and any abnormalities.

On postnatal day 63-67, up to 10 animals/sex/group were euthanized, by i.p. injection of a barbiturate, and perfused with glutaraldehyde and paraformaldehyde (followed by immersion in glutaraldehyde and paraformaldehyde) for brain weight measurements and/or neuropathology. The brain was transected from the spinal cord above the first cervical spinal nerve. The brain length was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum. The width was measured at the widest part of the cerebral hemispheres, and the brain was weighed. Animals were also subjected to macroscopic necropsy; abnormal tissues were preserved and livers and kidneys were weighed. The following central and peripheral nervous tissues (X) were dissected and preserved in plastic (sciatic and tibial nerves only) or paraffin (all other tissues).

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

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
x	**Coronal sections:** olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, tectum, tegmentum, medulla oblongata	x x	Mid-thigh** Sciatic Notch**
x	**Mid-sagittal sections**: cerebellum, pons		
	SPINAL CORD		**OTHER**
x	Cervical swelling**		Sural Nerve
x	Lumbar swelling**	x	Tibial Nerve (knee and calf muscle branch)**
			Peroneal Nerve
		x	Lumbar dorsal root ganglion*
		x	Lumbar dorsal root fibers*
	OTHER	x	Lumbar ventral root fibers*
	Gasserian Ganglion	x	Cervical dorsal root ganglion*
	Trigeminal nerves	x	Cervical dorsal root fibers*
x	Optic nerve*	x	Cervical ventral root fibers*
x	Eyes *	x	gastrocnemius muscle (transverse section)

* longitudinal sections
**longitudinal and transverse sections

Tissues from all dose groups were embedded; however, only brains were sectioned for mid- and low-dose animals. Paraffin-embedded tissues were sectioned at 4-5 ：m and stained with hematoxylin and eosin; plastic-embedded tissues were sectioned at 2 ：m and stained with toluidine blue. Tissues (listed below) from control and high-dose animals were examined microscopically.

Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was conducted as follows:

Thickness of the neocortex (distance from the pial surface to the top of the white matter was measured along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits the greatest thickness)

Corpus callosum (thickness at the midline)

Hippocampus (greatest dorsal-ventral thickness)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between the tip and base)

External germinal layer.

D. DATA ANALYSIS

1. **Statistical analyses:** Statistical analyses were performed on the following parameters: gestation body weight and body weight change, lactation body weight and body weight change, gestation food consumption, lactation food consumption, litter size, offspring survival indices, offspring body weight change, body weight change for offspring selected for behavioral testing, activity and rearing counts for dam FOBs, and FOB, activity counts, startle response, and water maze data for offspring.

   Methods: "If 75% of the data were the same value, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions and pairwise Fischer's Exact tests to compare each dose group to the control. If Bartlett's test for homogeneity was not significant at the 1% level, then parametric analysis was utilized. If the F1 test for monotonicity of dose-response was not significant at the 1% level, William's test for a monotonic trend was applied. If the F1 test was significant, Dunnett's test was applied."

   "If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then nonparametric tests were applied. If the H1 Test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H1 Test was not significant, Steel's test was performed."

   "If ANOVA was not significant, then significant results of inter-group comparison with the control are not reported."

2. **Indices:**

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

   Gestation index = (Number of live litters born/Number 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 No. of offspring born/Total No. of implantation sites) × 100

   Live birth index = (Number of live offspring at PND1/Total number of offspring born) × 100

   Viability index = (Number of live offspring at PND 4/Number of live offspring at PND1) ×100

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

[However, as noted below, the lactation index on day 21 was apparently calculated with the

number of live offspring on PND 11, prior to scheduled sacrifice, as the denominator.]

3. **Positive and historical control data:** Positive control data submitted by the laboratory are currently under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** There were no maternal deaths before scheduled termination except for early sacrifice due to litter loss. One litter in the 0.5 mg/kg/day group and three litters in the 3 mg/kg/day group were sacrificed during early lactation for reasons of animal welfare (due to clinical signs in the pups, see below); the dams were also sacrificed on the day of litter loss. General clinical signs in dams were limited to post-dosing salivation in 5 dams from the 0.5 mg/kg/day group and 2 dams from the 3 mg/kg/day group. In the 0.5 mg/kg/day group, this salivation was noted on day 7 of gestation for one dam, day 8 of gestation for 3 dams, and day 10 of gestation for 1 dam. This salivation was observed on day 6 of gestation in one 3 mg/kg/day animal and on days 11 and 14 of gestation in the other 3 mg/kg/day animal. Since these signs were observed only in mid- and high-dose animals, they may be treatment-related, however, given the sporadic incidence and lack of dose-response, this signs are not considered toxicologically significant.

   There were no substance-related functional observations. On post-natal days 4 and 10, slight salivation was noted in all treatment groups. Since this effect was noted in controls at a frequency and intensity similar to dose groups and since no dose-response relationship was observed, the effect is not considered test substance-related. There were no other treatment-related effects on cholinergic signs, such as urination or tremors.

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. There were no significant treatment-related effects on body weight or body weight gain during gestation (from the beginning of treatment on gestation day 6; we note there was a significantly lower weight gain in treated groups during gestation days 0-6, prior to the start of treatment) or lactation. The study authors noted that during postnatal days 1-4, 7 females in the 3 mg/kg/day group experienced a mean weight loss of 8 g, compared to a mean weight loss of 4 g in 2 control animals.

   There were no treatment-related effects on food consumption during gestation or lactation.

TABLE 2. Selected Mean (±SD) Maternal Body Weight and Food Consumption[a]

Observations/study interval	Dose (mg/kg/day)			
	0	0.1	0.5	3
Gestation (n= 23-24)				
Body wt. Gestation day 0 (g)	266±22	262±23	267±24	270±26
Body wt. Gestation day 6 (g)	301±21	292±22	297±24	301±30

TABLE 2. Selected Mean (±SD) Maternal Body Weight and Food Consumption[a]				
Observations/study interval	Dose (mg/kg/day)			
	0	0.1	0.5	3
Body wt. Gestation day 14 (g)	345±26	333±24	339±25	342±34
Body wt. Gestation day 20 (g)	428±35	414±30	421±32	421±43
Wt. gain gestation days 0-6 (g)	35±7	30±6*	30±5*	31±7*
Wt. gain gestation days 6-20 (g)	127±17	122±13	124±13	120±19
Food consumption gestation days 0-2 (g/animal/day)	28±3	27±2	29±3	28±3
Food consumption gestation days 6-9 (g/animal/day)	30±3	29±3	30±3	30±4
Food consumption gestation days 17-19 (g/animal/day)	31±3	29±2	30±3	30±3
Lactation (n=21-24)				
Body wt. lactation day 1(g)	333±27	321±26	327±29	325±38
Body wt. lactation day 11(g)	361±25	349±29	356±25	352±34
Body wt. lactation day 21(g)	357±32	347±23	357±25	359±38
Wt. gain lactation days 1-11 (g)	29±12	28±11	29±10	27±12
Wt. gain lactation days 1-21 (g)	25±20	26±10	30±12	34±14
Food consumption lactation days 1-10 (g/animal/day)	49±5	48±3	49±6	47±6
Food consumption lactation days 11-20 (g/animal/day)	73±7	70±6	70±10	69±13

[a] Data obtained from Tables 10-15 pages 87-92, MRID 45529703. Means during lactation exclude dams with total litter loss.
*p<0.05, compared with control mean.

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. There were no treatment- related effects on length of gestation, gestation index, the parturition process, or implantation rate. As noted above, there was no indication of treatment-related maternal toxicity or clinical signs, which might contribute to a lack of maternal care; signs indicative of poor maternal care were not noted in the study report (this issue is further discussed in the trip report from the GLP audit, TXR No. 014502).

TABLE 3. Reproductive Performance [a]				
Observation	Dose (mg/kg/day)			
	0	0.1	0.5	3
Number mated	24	24	24	24
Number Pregnant	24	23	24	24
Litter killed before weaning	0	0	1	3
Mean gestation duration (days)	22.2	22.1	22.2	22.1
Mean (±SD) implantations/dam	16.1±2.5	15.6±1.6	15.8±2.2	16.0±2.7
Gestation index (%)	100	100	100	100

[a]Data obtained from Tables 1,16, &19, pages 78, 93, &96, MRID 45529703.

4. **Maternal postmortem results:** No treatment-related effects were noted upon macroscopic examination at necropsy. There were no treatment-related effects on absolute or relative brain weight. The dams of the litters (one from the 0.5 mg/kg/day group and three from the

3 mg/kg/day groups) that were killed for reasons of animal welfare were also sacrificed on the day of litter loss. The mammary tissue of all of these dams was observed to be pale and inactive at necropsy.

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Tables 4a and 4b. There was no difference in litter size among groups at birth (Table 4a), but there was a decrease in pup survival in the mid- and high-dose groups (Table 4b). The decrease in survival became evident during early lactation and was most pronounced between PND1 and PND11.

   Consistent with the similar litter sizes among groups at birth, there were no treatment-related effects on the post-implantation survival index or sex ratio. The live birth index was significantly ($p<0.05$) reduced at 3 mg/kg/day compared to controls. The viability index is also decreased (82.7% for high dose, compared to 97.6% in controls), as are the lactation indices on days 7 and 11; these differences are not statistically significant. The decreased survival is no longer evident in the day 21 index (possibly due to changes in the calculation of the lactation index on day 21, as well as the inclusion of pups sacrificed for neuropathology on day 11 as pup deaths, see Table 4a footnote). The lack of significant differences in these indices may reflect: (1) the large variability in these indices among litters, (2) exclusion of litters with total pup loss from the mean calculations, and (3) inclusion of pups lost to scheduled sacrifice as pup deaths on day 11 (see table footnotes; the sacrifice on day 11 is not included in the pup mortality tabulated in Table 4b).

TABLE 4a. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	0	0.1	0.5	3
Number of litters born	24	23	24	24
Total number born	371	343	360	366
Number missing from day 1 count	1	4	5	8
Sex Ratio Day 1 (% %)	48.6	45.3	47.9	50
Total litter size	15.0±2.6	14.0±1.6	15.0±2.0	15.3±2.5
Mean litter size:†				
Day 1	14.9±2.6	14.7±1.7	14.8±2.0	14.9±2.4
Day 4 [b]	14.5±2.5	14.6±1.8	14.3±1.8	13.5±3.7
Day 4 [c]	8.0±0.0	8.0±0.0	8.0±0.0	7.7±1.1
Day 11	7.9±0.3	7.9±0.5	7.6±1.2	7.3±1.3
Day 17	6.8±0.5	6.9±0.5	6.6±1.1	6.3±1.2
Day 21	6.8±0.5	6.9±0.5	6.6±1.1	6.2±1.5
Post-implantation survival index (%)	92.9	95.6	94.6	95.5
Live birth index (%)	99.7	98.7	98.7	97.9*
Viability index	97.6	98.8	92.9	82.7
Lactation index Day 7	99	98.9	96.7	93
Day 11	98.4	98.4	95.1	88.2
Day 21‡	86.2	87.2	87	84

aData obtained from Tables 18-20, pages 95-97, and Appendix 8, pages177-180, MRID 45529703.
bBefore standardization (culling).
cAfter standardization (culling).
* Statistically different from control, $p<0.05$
†litters with total pup loss were excluded from mean litter size calculations; for 0.5 mg/kg/day group, n=23 from day 4 through day 21; for or 3.0 mg/kg/day group n=22 on day 4, n=21 from day 11 to day 21.
‡Lactation index for day 21 was calculated using day 11 (prior to the scheduled sacrifice) as the baseline, thus animals sacrificed for neuropathological evaluation are included as deaths in this calculation, but deaths prior to day 11 are not included.

The total number of pup deaths in each dose group, as well as the time frame in which the deaths occurred, are detailed in Table 4b. The increase was dose-related, with approximately 3 times as many deaths at 0.5 mg/kg/day, and 6 times as many deaths at 3.0 mg/kg/day, when compared to the control group. In addition to an increase in sporadic pup deaths during lactation, there was also a dose-related increase in total litter loss; one at 0.5 mg/kg/day and 3 at 3.0 mg/kg/day (there were no total litter losses in controls or at 0.1 mg/kg/day). At 3 mg/kg/day, one litter was sacrificed on postnatal day 3 and two litters were sacrificed on postnatal day 4. In the litter sacrificed on postnatal day 3, all offspring were cold to the touch and underactive with little food in the stomach on postnatal days 2 and 3. In one litter sacrificed on postnatal day 4, all offspring were small and underfed, and in the other litter sacrificed on postnatal day 4, all offspring were cold to the touch, underactive and had little food in the stomach. At 0.5 mg/kg/day, one litter was sacrificed on postnatal day 2; all offspring in this litter were cold to touch, underactive, and had little food in the stomachs.

As noted above, most of the pup deaths occurred during early lactation (PNDs 1-4 and 5-11 as shown in Table 4b), prior to the start of direct pup dosing. No excess pup deaths were noted during the period of direct dosing (PNDs 11-21).

Table 4b. Postnatal Pup Mortality [a]

Dose (mg/kg/day)	Days of Lactation					Total litter loss (day)	Mean number of dead pups/litter
	1-4	5-11	12-16	17-21	1-21		
0 (Control)	10 (7)	3 (3)	2 (2)	0 (0)	15 (10)	0	0.6
0.1 (LDT)	8 (5)	3 (2)	0 (0)	0 (0)	11 (6)	0	0.5
0.5 (MDT)	32* (9)	10 (3)	1 (1)	0 (0)	43* (10)	1 (2)	1.8
3.0 (HDT)	71* (13)	15 (7)	1 (1)	2 (2)	89* (14)	3 (3,4,4)	3.7

Number of pups (number of litters) [a] Includes pups that were found dead, missing and presumed dead, or sacrificed due to poor condition. * $p<0.01$, Chi Square test.

Reported clinical signs indicate that all offspring from 6 litters in the 3 mg/kg/day group exhibited signs of poor general condition or retarded development, such as small size, cold to touch, underfed, and/or underactive during early lactation. (Three of these litters were sacrificed on postnatal days 3 or 4 as described above). Additionally, all offspring in two additional litters in the 3 mg/kg/day group were small, underfed, cold to touch and underactive and were sacrificed at or shortly after weaning. No treatment-related clinical signs were noted at 0.5 mg/kg/day except those described above, and no treatment-related clinical signs were noted at 0.1 mg/kg/day.

2. **Body weight:** No treatment-related effects on body weight were observed at 0.1 or 0.5 mg/kg/day. Although statistical significance was not achieved, mean body weights at 3 mg/kg/day were slightly lower than controls for male (7.6-10%) and female (8.2-10.7%) offspring from postnatal days 4-21. The difference in body weight gain was most pronounced during days 1-4, corresponding to the period during which most pup deaths occurred. There was no apparent change in the pattern of body weight gain during the period of direct pup dosing (PND11-PND21). Selected mean preweaning pup body weight data are presented in Table 5.

TABLE 5. Mean (±SD) Pre-weaning Pup Body Weights and Body weight Gain (g) [a]								
Postnatal Day	Dose (mg/kg/day)							
	0	0.1	0.5	3	0	0.1	0.5	3
	Males				Females			
1	6.5±0.6	6.4±0.5	6.5±0.6	6.5±0.7	6.2±0.5	6.0±0.5	6.0±0.7	6.1±0.7
4 b	9.0±1.2	8.5±0.7	9.2±1.4	8.1±1.7 (10%)[d]	8.5±1.2	8.1±0.7	8.7±1.4	7.7±1.7 (9.4%)[d]
4 c	9.0±1.1	8.5±0.6	9.3±1.4	8.1±1.7 (10%)[d]	8.5±1.2	8.1±0.8	8.8±1.5	7.8±1.7 (8.2%)[d]
11	24.2±3.3	23.7±2.2	24.7±4.2	21.9±5.6 (9.5%)[d]	23.2±3.4	22.8±2.1	23.3±4.3	21.3±5.6 (8.2%)[d]
17	41.9±4.2	40.9±3.1	41.9±5.6	37.9±8.6 (9.5%)[d]	41.0±4.3	39.4±2.8	40.6±6.1	36.6±9.1 (10.7%[d])
21	52.3±5.8	50.9±4.0	52.8±6.6	48.3±11.1 (7.6%)[d]	51.5±6.0	48.9±3.5	51.0±7.2	47.1±11.2 (8.5%)[d]
Weight gain Days 1-4	2.4±0.9	2.2±0.6 (92%)[e]	2.8±1.1 (117%)[e]	1.7±1.4 (71%)[e]	2.3±1.0	2.1±0.8 (91%)[e]	2.7±1.0 (117%)[e]	1.7±1.4 (74%)[e]
Weight gain Days 1-11	17.6±3.1	17.3±2.2 (98%)[e]	18.2±3.9 (103%)[e]	15.5±5.4 (88%)[e]	17.1±3.2	16.8±2.1 (98%)[e]	17.3±3.9 (101%)[e]	15.3±5.3 (89%)[e]
Weight gain Days 11-21	28.1±3.1	27.2±2.4 (97%)[e]	28.1±3.0 (100%)[e]	26.4±6.0 (94%)[e]	28.3±3.4	26.1±2.0 (92%)[e]	27.7±3.3 (98%)[e]	25.4±6.3 (90%)[e]
Weight gain Days 1-21	45.7±5.6	44.6±4.0 (98%)[e]	46.3±6.3 (101%)[e]	41.8±10.9 (91%)[e]	45.4±5.8	42.9±3.4 (94%)[e]	45.0±6.8 (99%)[e]	41.0±11.0 (90%)[e]

a Data obtained from Tables 21-24, pages 98-101, MRID 45529703. n=20-24
b Before standardization (culling).
c After standardization (culling).
d % decrease compared to controls, calculated by reviewer
e % of control, calculated by reviewer

No treatment-related effects on postweaning body weights were observed. Selected mean postweaning offspring body weight data are presented Table 6.

TABLE 6. Mean (±SD) Post-weaning Pup Body Weights (g) [a]								
Postnatal Day	Dose (mg/kg/day)							
	0	0.1	0.5	3	0	0.1	0.5	3
	Males				Females			
35	139.5±17.4	139.3±11.7	142.3±17.9	139.0±19.2	124±14.3	120.9±11.1	123.3±13.3	123.4±10.9
49	270.0±29.0	270.1±24.1	276.2±31.2	266.6±35.3	194.1±21.1	189.9±16.3	194.1±18.8	193.6±13.9
56	334.1±33.0	334.7±29.2	342.5±36.4	332.2±39.2	222.5±22.4	218.2±18.8	223.6±21.2	219.9±14.5
63	381.9±36.1	384.2±34.4	392.3±42.1	383.1±41.6	243.8±25.2	239.3±20.2	244.0±23.7	240.0±16.2

a Data obtained from Tables 50 & 52, pages 131 &133, MRID 45529703. n=55-71

3. Developmental landmarks:

a. Sexual maturation: There were no treatment-related effects on the mean age for attainment

of vaginal opening for females or preputial separation for males. The data are presented in Table 7. Body weights at sexual maturation were also similar among groups.

TABLE 7. Mean (±SD) Age of Sexual Maturation (days) [a]

Parameter	Dose (mg/kg/day)			
	0	0.1	0.5	3
N (M/F)	69/71	65/71	66/66	52/55
Preputial separation (males)	46.0±2.8	45.6±2.4	46.0±2.8	46.0±3.0
Vaginal opening (females)	34.0±1.8	34.3±1.7	34.3±1.9	34.2±1.7

a. Data obtained from Tables 54 & 55, pages 135 &136, MRID 45529703

4. Behavioral assessments:

a. Functional observational battery: Data are summarized in Table 8. Males and females from the 3 mg/kg/day group were less active than controls on postnatal day 4 as shown by decreased number of sections entered, and supported by decreased maximum distance traveled, and maximum pivoting angle. Decreases seen in these measures in 0.5 mg/kg animals were less consistent. Surface righting was slowed somewhat in males and females in the 3 mg/kg/day group on postnatal day 11. Activity scores on PND 11 were decreased ($p<0.05$) in females at 0.1 and 3 mg/kg/day; however, the lack of an effect at 0.5 mg/kg/day makes this effect toxicologically questionable. Males (-62%; $p<0.01$) and females (-41%; $p<0.01$) in the 3 mg/kg/day groups had significantly decreased activity compared to controls on postnatal day 21. There were no treatment-related effects on FOB measures noted at postnatal days 35, 45, or 60.

TABLE 8. Functional Observational Battery Results (incidence) [a]				
	Dose (mg/kg/day)			
	0	0.1	0.5	3
Males				
Surface righting reflex (mean-scale of 1 to 3)				
-PND 4	2.7	2.4	2.5	2.3
-PND 11	1.2	1.5	1.3	1.9
Maximum pivoting angle (Mean)				
-PND 4	81	58.5	27	36
Maximum distance traveled (mean- cm)				
-PND 4	1.2	0.8	1.4	0.4
Activity (mean # of sections)				
-PND 4	2.6	2	1.3	0.7
Activity count (mean)				
-PND 11	2.5	1.8	2.6	2.9
-PND 21	7.1	4.7	4.5	2.7** (-62%)
-PND 35	11.9	11.6	12.2	9.7
-PND 45	6.4	7.9	7.1	5.5
-PND 60	7.0	12.3	10.0	8.5
Females				
Surface righting reflex (mean-scale of 1 to 3)				
-PND 4	2.5	2.6	2.5	2.9
-PND 11	1.4	1.4	1.3	1.7
Maximum pivoting angle (Mean)				
-PND 4	103.5	40.5	81	40.5
Maximum distance traveled (mean- cm)				
-PND 4	1.2	0.4	0.6	0.4
Activity (mean # of sections)				
-PND 4	2.6	1.3	1.9	0.9
Activity count (mean)				
-PND 11	3.0	1.3*	3.3	1.3*
-PND 21	7.4	7.8	6.3	4.4** (-41%)
-PND 35	13.2	11.8	14.3	13.5
-PND 45	13.0	15.6	15.0	11.6
-PND 60	15.4	19.2	15.9	14.1

a Data obtained from Tables 25-30, pages 102-107, MRID 45529703
N = 10/sex/dose
* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

b. Motor activity: Total activity data are presented in Tables 9 and 10 for rearing and horizontal activity, respectively.

The coefficients of variation for horizontal (cage floor) activity on PND 13 and PND 17 were roughly 100%, making statistical power and sensitivity low. On PND 13, high dose females showed an increase of 44%, while mid dose (0.5 mg/kg) females showed an increase of 113%. Neither difference was statistically significant. On PND 17, males showed dose dependent increases in horizontal activity of 43%, 65% and 122% compared to controls, respectively for low-, mid-, and high-dose groups. Females showed dose dependent *de*creases in horizontal activity on PND 17 of up to 42% at 3 mg/kg. Despite the lack of statistical significance, it is reasonable to consider the dose dependent increases on PND17 at 0.5 mg/kg (65%) and 3 mg/kg in males (122%) as treatment related. No effects on horizontal activity were noted on PND 22 and PND 59.

On PND 17, rearing was increased 104%, 154% and 98%, respectively for low-, mid-, and high-dose males. None were statistically significant. Mean rearing scores for females on postnatal day 17 were decreased non significantly at doses of 0.1 mg/kg/day (58%) and 0.5 mg/kg/day (43%), while at 3 mg/kg/day a 90% decrease was statistically significant ($p<0.01$). No consistent effects on rearing were noted at PND 13, 22, or 59.

Habituation of activity within the session became much more pronounced on postnatal days 22 and 59 in comparison to days 13 and 17. This is not unexpected. Females but not males showed the expected pattern of increases and then decreases in overall activity levels from day 13 to day 21.

TABLE 9. Mean (±S.D.) Motor Activity Data: Rearing (High Beam Breaks) (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)			
	0	0.1	0.5	3
Males				
PND 13	0.4±1.0	0.0±0.0	0.7±1.1	1.5±2.9
PND 17	12.3±16.3	25.1±38.5	31.3±68.8	24.4±29.0
PND 22	32.5±20.0	28.2±34.1	36.9±12.4	36.2±30.5
PND 59	247.9±67.3	276.6±110.2	228.7±84.5	257.6±141.7
Females				
PND 13	0.3±0.9	1.2±2.8	8.6±24.1	1.2±3.2
PND 17	46.4±56.3	19.5±20.3 (-58%)	26.5±23.6 (-43%)	4.5±8.1** (-90%)
PND 22	43.5±30.4	30.0±22.0	40.7±32.3	28.9±13.8
PND 59	272.1±132.4	281.9±96.1	281.1±118.8	318.1±166.2

a Data obtained from Tables 34-37, pages 111-118, MRID 45529703
N = 10/sex/dose
** Statistically different from control, $p<0.01$

TABLE 10. Mean (±S.D.) Motor Activity Data: Cage Floor Activity (Low Beam Breaks) (total activity counts for session) [a]				
Test Day	Dose (mg/kg/day)			
	0	0.1	0.5	3
Males				
PND 13	223.5±211.7	162.8±140.4	321.5±330.9	146.0±105.3
PND 17	171.1±147.2	244.6±231.3 (43%)	281.6±405.9 (65%)	379.9±407.0 (122%)
PND 22	198.1±85.0	199.6±117.9	235.2±107.3	196.4±97.0
PND 59	869.9±209.2	926.9±278.4	831.9±212.3	891.8±288.9
Females				
PND 13	153.6±138.1	205.8±138.8	328.6±431.0 (113%)	224.9±195.0 (44%)
PND 17	417.6±368.5	408.0±245.9	323.6±253.9	243.9±244.7 (-43%)
PND 22	249.2±142.2	192.7±127.7	240.0±154.9	211.9±108.9
PND 59	1220.3±379.9	1317.3±411.0	1151.2±260.6	1238.8±309.5

a Data obtained from Tables 34-37, pages 111-118, MRID 45529703
N = 10/sex/dose

c. <u>Auditory startle reflex habituation</u>: The auditory startle reflex peak amplitude data are shown in Table 11. Data on pre-pulse reflex inhibition are shown in Table 12. No consistent effects on peak amplitude in either sex at any dose was seen. No consistent differences in the degree of pre-pulse inhibition (roughly 25%) were seen in either sex at any dose.

TABLE 11. Auditory Startle Reflex Peak Amplitude Data (mean ±S.D.) [a]					
	Trial Block	Dose (mg/kg/day)			
		0	0.1	0.5	3
Males					
PND 23/24	1	175.6±72.5	171.4±53.8	182.4±67.8	173.9±55.0
	2	146.2±51.1	141.7±48.6	177.8±58.2	155.6±49.7
	3	144.8±57.9	129.4±36.9	165.8±73.1	159.0±77.5
	4	123.6±48.5	162.1±60.9	144.6±58.0	155.0±44.5
	5	129.6±58.5	148.1±52.3	154.9±77.5	148.4±76.5
PND 60/61	1	51.9±26.3	42.7±14.1	62.7±23.4	50.4±18.7
	2	45.1±23.9	34.7±11.5	55.6±13.5	46.7±15.8
	3	40.7±22.0	34.6±14.5	50.4±20.6	40.5±10.6
	4	37.2±23.2	35.2±9.4	44.4±16.4	38.6±15.5
	5	39.9±22.5	36.6±11.8	51.5±18.6	39.3±16.4
Females					
PND 23/24	1	170.7±67.5	194.0±71.5	165.4±116.8	142.6±56.5
	2	138.7±72.4	183.5±72.0	138.0±90.8	133.0±67.6
	3	125.8±57.1	159.9±74.0	150.0±86.2	115.1±63.1
	4	134.6±74.1	154.9±66.4	155.5±115.9	121.0±73.3
	5	142.5±74.6	151.2±52.0	159.7±132.6	130.9±66.7
PND 60/61	1	42.1±15.4	42.1±20.7	30.2±13.9	39.1±18.4
	2	36.5±17.7	42.6±21.2	26.5±12.4	30.0±11.6

TABLE 11. Auditory Startle Reflex Peak Amplitude Data (mean ±S.D.) [a]

	Trial Block	Dose (mg/kg/day)			
		0	0.1	0.5	3
	3	34.3±13.9	42.3±23.6	25.5±9.5	28.8±13.7
	4	26.9±14.6	38.2±28.5	29.2±21.6	26.8±8.8
	5	29.0±13.3	37.4±22.4	25.4±16.6	30.4±17.7

a Data obtained from Tables 42-45, pages 123-126, MRID 45529703
N = 9-10/sex/dose

TABLE 12. Auditory Startle Reflex Pre-Pulse Inhibition Peak Amplitude Data (mean ±S.D.) [a]

		Dose (mg/kg/day)			
		0	0.1	0.5	3
Males					
PND 23/24	Stimulus without pre-pulse	268.5±68.7	257.7±131.4	255.6±66.1	241.4±95.2
	Stimulus with pre-pulse	207.9±71.7	200.7±115.0	203.2±51.5	181.3±73.4
	% Inhibition	23.4±14.5	22.3±12.6	19.3±11.9	24.4±16.3
PND 60/61	Stimulus without pre-pulse	71.2±20.5	87.2±43.9	78.1±60.6	66.8±23.3
	Stimulus with pre-pulse	48.4±19.7	55.6±30.2	61.4±76.7	51.0±22.8
	% Inhibition	33.3±16.1	35.6±8.6	29.6±23.6	23.0±17.7
Females					
PND 23/24	Stimulus without pre-pulse	220.5±76.1	269.0±117.0	252.7±94.8	261.2±84.8
	Stimulus with pre-pulse	177.0±78.6	208.9±119.8	165.2±62.1	198.0±67.5
	% Inhibition	21.0±12.0	24.1±14.8	33.1±13.6	23.0±11.5
PND 60/61	Stimulus without pre-pulse	60.0±22.7	68.8±29.9	71.6±23.7	71.7±36.8
	Stimulus with pre-pulse	42.9±18.9	51.3±20.6	48.2±8.9	44.5±23.7
	% Inhibition	27.9±14.8	21.9±20.1	26.8±23.7	36.6±22.7

a Data obtained from Tables 46-49, pages 127-130, MRID 45529703
N = 9-10/sex/dose

d. <u>Learning and memory testing</u>: There were no treatment-related effects on performance in the Morris water maze at either time point. At PND 23/24 and at PND 61/62, animals demonstrated improved performance, as measured by trial time, failed trials, and number of sector entries, across the four test days. Initial performance levels and rates of learning were similar for all treatment groups. Data are summarized in Tables 13 and 14.

TABLE 13. Morris Water Maze Performance -Males (mean ± S.D.) [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.1	0.5	3
PND 23/24					
Test day 1	Trial time (sec)	65.5±22.3	59.2±21.1	59.8±20.3	61.5±15.1
	No. failed trials	1.5±1.2	1.1±0.9	1.3±1.1	1.4±0.9
	No. sector entries	16.9±6.0	16.6±5.0	15.5±4.7	16.4±4.1
Test day 2	Trial time (sec)	51.2±20.9	39.3±18.5	44.7±26.0	41.4±26.1
	No. failed trials	0.9±0.9	0.4±0.7	0.7±1.1	0.6±1.1
	No. sector entries	14.6±5.6	11.7±4.9	11.9±5.6	13.2±7.2
Test day 3	Trial time (sec)	36.2±26.1	26.6±11.2	39.3±20.4	35.6±16.0
	No. failed trials	0.7±1.1	0.1±0.3	0.5±0.8	0.2±0.4
	No. sector entries	10.5±6.1	8.5±2.3	10.9±5.2	10.4±4.4
Test day 4	Trial time (sec)	23.2±13.2	18.9±11.9	20.3±7.5	24.1±12.0
	No. failed trials	0.1±0.3	0.0±0.0	0.0±0.0	0.2±0.4
	No. sector entries	7.9±3.6	6.7±3.5	6.7±1.4	6.9±3.3
PND 61/62					
Test day 1	Trial time (sec)	71.8±18.2	61.0±17.9	68.0±19.8	71.2±15.2
	No. failed trials	1.8±1.0	1.2±0.8	1.7±1.1	1.7±0.9
	No. sector entries	15.4±3.7	13.5±3.2	15.0±4.3	16.3±3.2
Test day 2	Trial time (sec)	30.8±16.1	31.7±18.3	47.7±24.6	39.2±20.7
	No. failed trials	0.2±0.6	0.1±0.3	0.7±0.8	0.3±1.0
	No. sector entries	8.7±3.1	9.4±4.6	12.3±5.7	10.5±4.3
Test day 3	Trial time (sec)	24.5±15.5	21.1±21.3	18.1±14.4	25.5±15.8
	No. failed trials	0.2±0.4	0.1±0.3	0.0±0.0	0.0±0.0
	No. sector entries	7.4±3.9	6.0±4.6	6.1±3.3	7.6±4.6
Test day 4	Trial time (sec)	20.6±13.4	23.4±17.7	24.5±25.1	25.3±16.8
	No. failed trials	0.0±0.0	0.2±0.6	0.3±0.9	0.1±0.3
	No. sector entries	6.7±4.4	6.0±3.2	7.4±6.0	7.0±3.2

a Data obtained from Tables 38 & 40, pages 119 & 121, MRID 45529703
N = 9-10/sex/dose

TABLE 14. Morris Water Maze Performance -Females (mean ± S.D.) [a]

Test Day/Parameter		Dose (mg/kg/day) 0	0.1	0.5	3
PND 23/24					
Test day 1	Trial time (sec)	57.5±14.4	69.8±18.2	68.1±17.4	60.9±19.1
	No. failed trials	1.2±0.4	1.3±0.9	1.9±0.9	1.1±1.1
	No. sector entries	16.0±4.1	18.3±4.0	17.5±2.9	16.5±4.3
Test day 2	Trial time (sec)	37.5±23.5	49.3±20.8	42.4±21.1	36.5±22.4
	No. failed trials	0.5±0.8	0.8±0.9	0.5±0.8	0.4±0.5
	No. sector entries	11.4±6.1	13.8±6.0	12.8±4.6	10.1±4.4
Test day 3	Trial time (sec)	39.5±17.9	33.8±13.8	28.2±13.0	33.9±18.2
	No. failed trials	0.4±0.5	0.3±0.5	0.3±0.5	0.3±0.7
	No. sector entries	12.2±5.7	9.8±4.4	9.2±4.2	10.0±5.2
Test day 4	Trial time (sec)	22.7±12.0	27.7±13.3	25.1±13.7	20.9±9.1
	No. failed trials	0.1±0.3	0.2±0.4	0.3±0.5	0.0±0.0
	No. sector entries	7.8±4.6	8.2±3.0	8.3±3.9	7.4±3.4
PND 61/62					
Test day 1	Trial time (sec)	69.8±18.9	66.9±12.4	77.9±15.0	66.9±14.0
	No. failed trials	1.7±1.1	1.7±0.7	2.2±0.8	1.5±1.0
	No. sector entries	17.1±5.0	15.3±2.8	17.3±3.1	15.7±4.1
Test day 2	Trial time (sec)	41.9±21.4	39.1±16.0	40.8±16.9	42.1±24.2
	No. failed trials	0.4±1.0	0.3±0.5	0.4±0.7	0.5±0.8
	No. sector entries	11.7±4.1	11.2±4.4	10.4±2.9	10.9±5.4
Test day 3	Trial time (sec)	18.7±14.1	22.5±15.7	29.7±20.6	16.4±9.6
	No. failed trials	0.1±0.3	0.2±0.4	0.2±0.6	0.0±0.0
	No. sector entries	6.1±3.7	6.8±3.8	8.9±5.9	4.9±2.2
Test day 4	Trial time (sec)	14.1±8.6	25.6±17.0	30.6±18.8	20.5±14.4
	No. failed trials	0.0±0.0	0.2±0.4	0.4±0.7	0.1±0.3
	No. sector entries	5.1±2.2	7.2±3.6	8.0±4.2	6.3±4.2

a Data obtained from Tables 39 & 41, pages 120 & 122, MRID 45529703
N = 9-10/sex/dose

5. Postmortem results:

1) Unscheduled deaths: Necropsies were conducted on some of the F1 animals that died or were sacrificed for humane reasons prior to scheduled sacrifice. Although there were scattered findings, including eyes opaque or bilateral renal cavitation, none appeared to be common among treated animals.

2) Animals selected for neuropathology

a. Brain weights: There were no treatment-related effects on absolute or relative brain weights in male or female offspring at postnatal days 11, 21, or 63-67. Mean brain weight data (for animals selected for neuropathology) are presented in Table 15.

TABLE 15. Mean (±SD) Brain Weight Data in Offspring [a]				
Parameter	Dose (mg/kg/day)			
	0	0.1	0.5	3
Males				
Day 11				
Terminal body weight (g)	24.5±4.6	24.5±2.0	25.2±3.3	24.7±2.0
Brain weight (g)	1.159±0.140	1.160±0.076	1.164±0.064	1.194±0.073
Brain-to-body weight ratio	4.811±0.520	4.742±0.255	4.678±0.485	4.844±0.278
Day 21				
Terminal body weight (g)	51.8±5.6	50.7±6.3	50.5±8.8	50.2±7.2
Brain weight (g)	1.251±0.089	1.297±0.088	1.207±0.065	1.282±0.063
Brain-to-body weight ratio	2.437±0.300	2.581±0.215	2.476±0.551	2.599±0.382
Days 63-67				
Terminal body weight (g)	402.0±27.9	417.9±38.6	428.4±36.2	405.6±33.4
Brain weight (g)	1.690±0.128	1.798±0.176	1.735±0.112	1.804±0.131
Brain-to-body weight ratio	0.422±0.035	0.433±0.049	0.403±0.042	0.447±0.038
Females				
Day 11				
Terminal body weight (g)	21.7±5.2	22.1±2.5	24.5±3.9	19.0±6.0
Brain weight (g)	1.066±0.143	1.137±0.089	1.153±0.074	0.994±0.213
Brain-to-body weight ratio	5.093±0.833	5.182±0.540	4.774±0.536	5.508±0.964
Day 21				
Terminal body weight (g)	52.1±5.0	49.8±4.5	52.7±6.7	51.6±4.1
Brain weight (g)	1.238±0.089	1.271±0.101	1.141±0.113	1.220±0.066
Brain-to-body weight ratio	2.392±0.247	2.564±0.232	2.187±0.262	2.378±0.221
Termination				
Terminal body weight (g)	253.0±12.7	245.7±23.0	255.8±20.8	254.6±19.6
Brain weight (g)	1.603±0.109	1.596±0.108	1.575±0.190	1.666±0.077
Brain-to-body weight ratio	0.633±0.066	0.655±0.081	0.623±0.104	0.658±0.054

a Data obtained from pages Tables 56, 57 & 61, pages 137, 138, & 143, MRID 45529703
N = 8-13/sex/dose

Brain weights were also evaluated for non-perfused animals sacrificed at day 65 (n=43/46, 56/56, 55/61, and 59/61 [M/F] for control, low, mid, and high dose groups, respectively). Consistent with the results for neuropathology animals, brain weights were similar across all treatment groups in these additional animals.

b. Neuropathology

1. **Macroscopic examination:** No treatment-related effects were reported for male or female offspring at postnatal days 11, 21, or 63-67.

2. **Microscopic examination:** No significant treatment-related effects were noted on postnatal

days 21 or 63-67. On postnatal day 21, there was a minimal focus of degeneration of the granular layer of the cerebellum of one female in the 3 mg/kg/day group. There was also a malformation of the cerebellar folia of one control male and one high-dose female on day 21.

On postnatal day 63-67, there was a minimal focus of degeneration of the granular layer of the cerebellum of one control male and one control female. Minimal or slight degenerative changes were observed in the peripheral nerves of control and high-dose animals. This degeneration was observed in similar incidences and severity in control and high-dose animals. There was also a single incidence of mammary adenocarcinoma in one high-dose female at terminal sacrifice. These findings are considered incidental to treatment.

There were no differences in brain length or width in males or females on postnatal days 21 or 63-67. Data are summarized in Table 16.

TABLE 16. Mean (±SD) Brain Length and Width Data for Offspring [a]

Parameter	Dose (mg/kg/day)			
	0	0.1	0.5	3
Males				
Day 21				
Brain length (mm)	17.9±0.5	17.9±0.5	17.5±0.6	17.9±0.6
Brain Width (mm)	14.5±0.3	14.5±0.6	14.7±0.8	14.5±0.3
Termination				
Brain length (mm)	20.9±0.8	21.0±0.5	21.1±0.9	20.9±0.3
Brain Width (mm)	15.0±0.6	15.0±0.6	15.6±0.4	15.4±0.4
Females				
Day 21				
Brain length (mm)	17.7±0.4	17.7±0.5	17.2±0.9	17.6±0.4
Brain Width (mm)	14.3±0.6	14.1±0.6	14.5±0.4	14.6±0.3
Termination				
Brain length (mm)	20.5±0.6	20.0±0.4	20.4±0.6	20.4±0.7
Brain Width (mm)	14.9±0.4	14.8±0.6	14.9±0.5	15.1±0.2

a Data obtained from pages Tables 58 & 62, pages 139 & 145, MRID 45529703
N = 8-10/sex/dose

There were no differences among treatment groups in morphometric measurements for males or females on postnatal days 21 or 63-67. Data are summarized in Table 17.

TABLE 17. Mean (±SD) Morphometric Data for Offspring [a]		
Parameter	Dose (mg/kg/day)	
	0	3
Males		
Day 21		
Neocortex (mm)	1.63±0.19	1.62±0.11
Hippocampus (mm)	1.67±0.14	1.55±0.16
Corpus Callosum (mm)	0.22±0.10	0.17±0.04
Cerebellum (mm)	0.68±0.09	0.71±0.10
Termination		
Neocortex (mm)	1.77±0.17	1.71±0.08
Hippocampus (mm)	1.77±0.17	1.86±0.26
Corpus Callosum (mm)	0.28±0.08	0.33±0.14
Cerebellum (mm)	0.80±0.08	0.87±0.08
Females		
Day 21		
Neocortex (mm)	1.68±0.14	1.72±0.10
Hippocampus (mm)	1.62±0.08	1.63±0.20
Corpus Callosum (mm)	0.19±0.06	0.19±0.05
Cerebellum (mm)	0.71±0.04	0.68±0.05
Termination		
Neocortex (mm)	1.69±0.15	1.70±0.11
Hippocampus (mm)	1.76±0.24	1.83±0.17
Corpus Callosum (mm)	0.21±0.04	0.23±0.07
Cerebellum (mm)	0.78±0.10	0.81±0.05

a Data obtained from pages Tables 59 & 63, pages 140 & 146, MRID 45529703
N = 8-10/sex/dose

c. Pathological evaluations of additional tissues

Histopathological evaluation of selected additional tissues in PND21 offspring revealed similar findings in treated and control animals. The most common findings were cortical tubular dilatation and vacuolation in the kidneys; these lesions occurred with similar frequency in control and high dose animals.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that there was no selective developmental neurotoxicity at the high dose of 3 mg/kg/day, and that the slight developmental delay prior to weaning occurred in the context of increased litter/pup mortality, signs of poor general condition and decreased early weight of pups. The investigators considered 0.5 mg/kg/day Dimethoate as a NOAEL for morphological and

functional development.

B. REVIEWER COMMENTS: There were no treatment-related effects for maternal animals. Clinical signs, reproductive parameters through gestation, body weights, and food consumption were similar across all treatment groups. The maternal LOAEL is not identified. The maternal NOAEL is 3 mg/kg/day.

There were no differences among treatment groups with respect to pup body weight or food consumption, auditory startle parameters, learning and memory evaluations, brain weights and measurements, or histopathological evaluations at either time point. Although body weights were slightly reduced at 3.0 mg/kg/day in pups, these differences were small and did not achieve statistical significance. The reductions occurred early during the lactation period; there was no indication of increased pup toxicity during the direct dosing period (PND 11-21).

Pups in the 3 mg/kg group showed some general decreases in activity in the FOB between PND 4 and PND 21. On PND 4, males and females were less active, as seen in number of sections entered in an open field, as well as decreased distance traveled, and maximum pivoting angle. On PND 11, males and females had somewhat slowed righting responses; and on PND 21 males and females had significantly decreased activity in the open field. These differences, seen only at the high dose, are considered treatment-related.

Changes in automated motor activity measures were seen only on PND 17, and consisted of a significant decrease in rearing in females given 3 mg/kg, and large, but not statistically significant, increases in horizontal activity in males at 0.5 mg/kg (65%) and 3.0 mg/kg (122%). These differences are considered treatment-related at the mid- and high-doses.

The strongest finding in the study was the dose-related increase in pup deaths occurring at the 0.5 and 3.0 mg/kg/day doses. In addition to sporadic pup deaths scattered among litters, there were three incidences of total litter loss at 3.0 mg/kg/day and one total litter loss at 0.5 mg/kg/day. Most of the pup deaths occurred during early lactation (days 1-11), although sporadic deaths continued in the 3.0 mg/kg dose group throughout lactation and surviving pups from two additional litters died shortly after the lactation period ended. Historical control data on litter loss, provided during the study audit, are discussed in the trip report cited earlier (TXR #014502). Data from three additional studies, provided as Attachment 10 (p. 639) in the study report, indicate no incidences of total litter loss from control groups in those studies (conducted in October 2000 and April 2001). The total number of pups found dead in those studies was also provided; the largest number, 23 pups found dead in a study with "an increased level of monitoring of litters" was lower than the number of pups lost in the mid-dose group of the current study (43), although similar to the number of pups lost when the total litter loss was excluded (24 pups for the current study). It is unclear what is meant by "an increased level of monitoring of litters," but the next highest number of lost pups (15) was identical to the number of lost pups in the control group of the current study (15).

Based on the available information, we believe the pup deaths to be treatment-related at both

the mid- and high-dose. Although no increase in pup deaths was seen in the companion cholinesterase inhibition study (MRID 45529702, reviewed separately), conducted using the same doses as the current study, the sample size in that study was much smaller than the current study (10 litters/dose, as opposed to 24 in the current study). No increase in pup deaths was seen at 3.0 mg/kg/day in the range-finding study (MRID 45529701, reviewed separately), which also included 10 litters/dose, however there was large increase in pup death at the higher dose tested in that study (6.0 mg/kg/day). Similar to what was seen in the current study, most of the deaths at 6.0 mg/kg/day occurred during early lactation (days 1-4), and there were two instances of total litter loss.

In the companion cholinesterase inhibition study, repeated administration of dimethoate led to cholinesterase inhibition at doses of 0.5 mg/kg/day and 3.0 mg/kg/day, in several compartments for dams and pups (following a single dose of dimethoate, cholinesterase inhibition was seen only at the 3.0 mg/kg dose). The level of cholinesterase inhibition seen in the companion study was similar for adults and pups receiving similar doses of dimethoate. Thus, the pup death seen in the current study was occurring at the same dose level which caused similar cholinesterase inhibition in both dams and pups in the companion study. We note, however, that the pup deaths in the current study were occurring at doses that caused no changes in clinical signs, body weight, or food consumption in dams; we believe that the pup death represents an increase in severity of the response to dimethoate in young vs. adult animals.

The offspring LOAEL is 0.5 mg/kg/day, based on increased pup death and increases in motor activity at that dose. The offspring NOAEL is 0.1 mg/kg/day.

C. <u>STUDY DEFICIENCIES:</u> The coefficients of variation for horizontal motor activity measurements in pre-weaning rats were 50-100%. This made changes that were seen in these measures more difficult to interpret, due to a lack of statistical significance. One approach that might have helped with analysis of this data would be to make use of repeated measures analyses of variance [See e.g., Tamura, R.N. and J. Buelke-Sam (1992) The use of repeated measures analysis in developmental neurotoxicity studies. *Neurotoxicol. Teratol.* 14:205-210].

Positive control data is currently under evaluation.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
		dev neurotox	rats		oral	diet						Maternal
		dev neurotox	rats		oral	diet						Offspring