11/09/2005

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR No. 0053832

MEMORANDUM

DATE: 11/03/2005

SUBJECT: Re-evaluation of DNT Studies for DDVP-Combined Studies Satisfy Guideline Requirement for Acceptable DNT

DP Barcode: D323156
PC Code: 084001

PRAT Case#:0310
MRID No.:46153302, 46239801, 46487401

TO: Dayton Eckerson and Eric Olson, Product Manager#61
Special Review Branch
Special Review and Reregistration Division (7509C)

FROM: William Dykstra, Ph.D., Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

William Dykstra
11/03/05

THRU: Susan Hummel, Branch Senior Scientist.
Reregistration Branch 4
Health Effects Division (7509C)

Susan Hummel

Background and Request: Based on the June 14, 2005 HED Chapter of the DDVP RED, the registrant for DDVP, AMVAC Chemical Corporation, requests in their July 29, 2005 response document that HED reconsider the unacceptable classification of the two DNT studies with dichlorvos (MRID 46153302, and 46239801) in light of recently submitted Historical Control Information (MRID 46487401). AMVAC states that EPA erred in finding the DNT study not acceptable because the finding does not take into account the fact that two studies, considered together, fully satisfy the Guideline requirement. The DNT Committee met on September 1, 2005 to consider the AMVAC request in light of the newly submitted Historical Control information (MRID 46487401).

Review:

1. The historical control information (MRID 46487401) supplied by AMVAC contained results of 29 DNT or multigeneration studies initiated in the period December 1996 to July 2003 in Alpk:APfSD (Wistar strain) either dosed by gavage or in the diet. Data considered were pups born alive, pup survival on day 5 (without litter losses), pup survival on day 5 (with whole litter losses included), incidence of litter losses in each study, and health monitoring investigations for rats at the breeding unit, health monitoring of rats in CTL (testing laboratory), prevention of possible infection at CTL, and investigation of environmental factors.

Conclusion. The DNT Committee determined that the high incidence of total litter losses, which resulted in poor day 5 pup survival in the two DNT studies with dichlorvos (77% survival in RR0886 [6 total litter losses/30 control females] and 73.7% survival in RR0988 [5/29]), both by gavage, resulted by chance due to random factors and not due to any deficiencies in the conduct of the study or environmental factors at CTL. Studies conducted both before , during or after the dichlorvos studies, either by diet or gavage, did not demonstrate any trends or patterns of distributions of litter losses or other unusual findings suggestive of any deficiencies at the CTL laboratory.

2. The DNT Committee next considered whether the two DNT studies could be combined and, if so, what would be the overall NOAEL/LOAEL for the combined studies and the overall developmental effects observed in the combined studies.

Conclusion: The DNT Committee determined that the two DNT studies combined had acceptable numbers of total pups examined in the controls and high dose groups (> 35 pups/sex in combined studies) and, therefore, the developmental results of the combined studies could be evaluated for the NOAEL/LOAEL. The classification of the studies was changed from unacceptable/non-guideline to **Acceptable/non-guideline.** A comparison of the developmental findings showed that the auditory startle reflex habituation Vmax in PND 23 high dose males in study RR0886 had **statistically significant increases** (39-47%) in 4 out of 5 blocks and study RR0988 **had increases** (7-15%), although not statistically significant, in this same Vmax parameter also in PND 23 high dose males in 5 out of 5 blocks in comparison to controls. Therefore, the developmental/offspring NOAEL was determined to be 1.0 mg/kg/day (based on study RR0886) and the LOAEL was 7.5 mg/kg/day (based on both studies RR0886 and RR0988) with the effect being increases in auditory startle amplitude in both studies.

3. The revised Executive Summaries for the two DNT studies are presented below:

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (2003, MRID 46153302, study RR0886) Dichlorvos (99.0% a.i., batch #ST120700) was administered to 30 time-mated female Alpk:AP$_f$SD. (Wistar-derived) rats per group by gavage in de-ionized water at dose levels of 0, 0.1, 1.0, or 7.5 mg/kg bw/day from gestation day (GD) 7 through postnatal day (PND) 7 and direct treatment of the F_1 offspring was carried out during PND 8-22, inclusive. On PND 5, litters were culled to 8

pups (4/sex as closely as possible), and litters containing fewer than 7 pups and/or fewer than 3 pups of each sex were removed from the study. The dams were subjected to a functional observational battery (FOB) on GDs 10 and 17 and on PNDs 2 and 9. The F_1 offspring were observed for attainment of preputial separation or vaginal patency. Animals were allocated from within litters for use in the following investigations: functional observational battery assessments (PNDs 5, 12, 22, 36, 46, and 61); locomotor activity assessment (PNDs 14, 18, 22, and 60); auditory startle habituation (PNDs 23 and 61), water maze testing (PND 24-27 or PND 59-62); and post mortem investigations including brain weight, neuropathology, and morphometry (PNDs 12 and 63). Dosing was based on a preliminary developmental neurotoxicity study in rats (MRID 46153301).

One high-dose female was sacrificed on LD 3 due to clinical signs (pallor, piloerection, and slightly hunched posture and thin appearance) and had a pale liver at necropsy. One mid-dose female died on GD 24 due to parturition difficulties. There were no treatment-related effects on maternal body weight, FOB parameters, or gestation length. **The maternal NOAEL is 7.5 mg/kg/day, the highest dose tested. A maternal LOAEL was not established.**

During LD 1-5, the control, low-, mid-, and high-dose groups, respectively, had pup mortality of 22.6, 17.4, 17.5, and 28.1%, and there were total litter losses of 20.0, 10.0, 17.9, and 18.5% of the litters in these same respective groups. There were 2 total litter resorptions in the high-dose group. The number of litters available which were used for F1 offspring was 23, 21, 21, and 14 and the viability indices were 77.4, 82.6, 82.5, and 69.0% for the control, low, mid, and high dose groups, respectively.

Due to the low number of pups available in the high dose group, it was necessary to combine this study (RR0886) with a repeat study (2004, MRID 46239801; study No. RR0988) consisting of controls and a dose level of 7.5 mg/kg in order to have sufficient pups for all assessments.

The DNT Committee determined that the two DNT studies combined (RR0886 and RR0988) had acceptable numbers of total pups examined in the controls and high dose groups (> 35 pups/sex examined in combined studies) and, therefore, the developmental results of the combined studies could be evaluated for the NOAEL/LOAEL. The classification of the studies taken together was changed from unacceptable/non-guideline to **Acceptable/non-guideline.** A comparison of the developmental findings showed that the auditory startle reflex habituation Vmax in PND 23 high dose males in study RR0886 had **statistically significant increases** (37-49%) in 4 out of 5 blocks and study RR0988 **had increases** (7-15%), although not statistically significant, in this same Vmax parameter in PND 23 high dose males in 5 out of 5 blocks in comparison to controls for each study.

Therefore, the developmental/offspring NOAEL was determined to be 1.0 mg/kg/day (based on study RR0886) and the developmental/offspring LOAEL was 7.5 mg/kg/day (based on both studies RR0886 and RR0988) with the effect being increases in auditory startle reflex habituation Vmax in PND 23 high dose males in both studies.

This study when combined with the accompanying study is classified **Acceptable/non-guideline** and may be used for regulatory purposes. It does satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] pending review of the positive control data.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2004, MRID 46239801, study RR0988) Dichlorvos (99.0% a.i., batch #ST120700) was administered to 30 time-mated female Alpk:AP$_f$SD (Wistar-derived) rats per group by gavage in de-ionized water at dose levels of 0 or 7.5 mg/kg bw/day from gestation day (GD) 7 through postnatal day (PND) 7. Direct dosing of the F_1 offspring was carried out during PNDs 8-22, inclusive. **This study was conducted with a single dose to provide supplemental information to the previous study (MRID No. 46153302) where high number of whole litter loss at this dose was seen.**

On PND 5, litters were culled to 8 pups (4/sex as closely as possible), and litters containing fewer than 7 pups and/or fewer than 3 pups of each sex were removed from the study. The dams were subjected to a functional observational battery (FOB) on GDs 10 and 17 and on PNDs 2 and 9. The F_1 offspring were observed for attainment of preputial separation or vaginal patency. Animals were allocated for assessment of FOB (PNDs 5, 12, 22, 36, 46, and 61), locomotor activity (PNDs 14, 18, 22, and 60), auditory startle reflex habituation (PNDs 23 and 61), learning and memory (PND 24-27 or PND 59-62), and post mortem investigations including brain weight, neuropathology, and morphometry (PNDs 12 and 63).

No treatment-related deaths, clinical signs of toxicity, or abnormal FOB findings were observed in any maternal animals during the study. Maternal body weight, pregnancy rate, and gestation length were similar between the treated and control groups.

The maternal NOAEL is 7.5 mg/kg/day, the highest dose tested. A maternal LOAEL was not established.

The results of this study were confounded again by excessive litter loss in the control group similar to that of the previous study. In the control group a total of five dams had complete litter loss during lactation and another eight litters had insufficient numbers of pups for selection of F_1 animals. Only two treated dams had complete litter loss. The reason for the pup mortality is unknown but was also seen at the same dose (7.5 mg/kg/day) in the previous study. Therefore, it appears that the pup mortality may not be related to treatment, but rather reflects a chance occurrence.

In the offspring available for evaluation, no treatment-related effects were observed on body weight, body weight gain, food consumption, developmental landmarks, FOB, motor activity, auditory startle reflex, learning and memory, brain weight, brain morphology or neuropathology.

The DNT Committee determined that the two DNT studies combined (RR0886 and RR0988) had acceptable numbers of total pups examined in the controls and high dose groups (> 35 pups/sex

examined in combined studies) and, therefore, the developmental results of the combined studies could be evaluated for the NOAEL/LOAEL.

Therefore, the developmental/offspring NOAEL was determined to be 1.0 mg/kg/day (based on study RR0886) and the developmental/offspring LOAEL was 7.5 mg/kg/day (based on both studies RR0886 and RR0988) with the effect being increases in auditory startle reflex habituation Vmax in PND 23 high dose males in both studies.

This study when combined with the accompanying study is classified **Acceptable/non-guideline** and may be used for regulatory purposes. It does satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] pending review of the positive control data.

DATA EVALUATION RECORD

DICHLORVOS/084001

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT
[OPPTS 870.6300 (§83-6); OECD 426]

MRIDs 46153302 (Main Study), 46153301 (Preliminary)

Prepared for

Health Effects Division
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Prepared by

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

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Template version 11/01

TXR#:0053832

DATA EVALUATION RECORD

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

PC CODE: 084001 **DP BARCODE:** D298913
SUBMISSION NO.: none provided

TEST MATERIAL (PURITY): Dichlorvos Technical Material (99.0% a.i.)

SYNONYMS: DDVP

CITATION: G. Milburn (2003) Dichlorvos: developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory report number CTL/RR0886/Regulatory/Report, November 10, 2003. MRID 46153302. Unpublished.

G. Milburn (2003) Dichlorvos: preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory report number CTL/RR00885/Regulatory/Report, October 13, 2003. MRID 46153301. Unpublished.

SPONSOR: Amvac Chemical Corporation.

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (2003, MRID 46153302, study RR0886) Dichlorvos (99.0% a.i., batch #ST120700) was administered to 30 time-mated female Alpk:AP$_f$SD. (Wistar-derived) rats per group by gavage in de-ionized water at dose levels of 0, 0.1, 1.0, or 7.5 mg/kg bw/day from gestation day (GD) 7 through postnatal day (PND) 7 and direct treatment of the F_1 offspring was carried out during PND 8-22, inclusive. On PND 5, litters were culled to 8 pups (4/sex as closely as possible), and litters containing fewer than 7 pups and/or fewer than 3 pups of each sex were removed from the study. The dams were subjected to a functional observational battery (FOB) on GDs 10 and 17 and on PNDs 2 and 9. The F_1 offspring were

observed for attainment of preputial separation or vaginal patency. Animals were allocated from within litters for use in the following investigations: functional observational battery assessments (PNDs 5, 12, 22, 36, 46, and 61); locomotor activity assessment (PNDs 14, 18, 22, and 60); auditory startle habituation (PNDs 23 and 61), water maze testing (PND 24-27 or PND 59-62); and post mortem investigations including brain weight, neuropathology, and morphometry (PNDs 12 and 63). Dosing was based on a preliminary developmental neurotoxicity study in rats (MRID 46153301).

One high-dose female was sacrificed on LD 3 due to clinical signs (pallor, piloerection, and slightly hunched posture and thin appearance) and had a pale liver at necropsy. One mid-dose female died on GD 24 due to parturition difficulties. There were no treatment-related effects on maternal body weight, FOB parameters, or gestation length. **The maternal NOAEL is 7.5 mg/kg/day, the highest dose tested. A maternal LOAEL was not established.**

During LD 1-5, the control, low-, mid-, and high-dose groups, respectively, had pup mortality of 22.6, 17.4, 17.5, and 28.1%, and there were total litter losses of 20.0, 10.0, 17.9, and 18.5% of the litters in these same respective groups. There were 2 total litter resorptions in the high-dose group. The number of litters available which were used for F1 offspring was 23, 21, 21, and 14 and the viability indices were 77.4, 82.6, 82.5, and 69.0% for the control, low, mid, and high dose groups, respectively.

Due to the low number of pups available in the high dose group, it was necessary to combine this study (RR0886) with a repeat study (2004, MRID 46239801; study No. RR0988) consisting of controls and a dose level of 7.5 mg/kg in order to have sufficient pups for all assessments.

The DNT Committee determined that the two DNT studies combined (RR0886 and RR0988) had acceptable numbers of total pups examined in the controls and high dose groups (> 35 pups/sex examined in combined studies) and, therefore, the developmental results of the combined studies could be evaluated for the NOAEL/LOAEL. The classification of the studies taken together was changed from unacceptable/non-guideline to **Acceptable/non-guideline.** A comparison of the developmental findings showed that the auditory startle reflex habituation Vmax in PND 23 high dose males in study RR0886 had **statistically significant increases** (37-49%) in 4 out of 5 blocks and study RR0988 **had increases** (7-15%), although not statistically significant, in this same Vmax parameter in PND 23 high dose males in 5 out of 5 blocks in comparison to controls for each study.

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This study when combined with the accompanying study is classified **Acceptable/non-guideline** and may be used for regulatory purposes. It does satisfy the guideline requirement for a

developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] pending review of the positive control data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlorvos

Description:	technical material; clear, colorless liquid
Batch #:	ST120700
Purity:	99.0 % a.i.
Compound Stability:	stability not reported; expiration date of October 22, 2003
CAS # of TGAI:	not reported
Structure:	not available

2. Vehicle and/or positive control: The vehicle was de-ionized water. No positive control was used in the current study.

3. Test animals (P):

Species:	Rat	
Strain:	Alpk:AP$_f$SD. (Wistar-derived)	
Age at study initiation:	approximately 10-12 wks	
Wt. at study initiation:	221-297 g	
Source:	Rodent Breeding Unit (RBU), Alderley Park, Macclesfield, Cheshire, UK	
Housing:	P: Individually in solid plastic cages with sawdust bedding; loose paper balls were provided as nesting materials (SI Supplies, Hazel Grove, Cheshire). F_1: in same sex groups of up to 4 animals in wire mesh cages	
Diet:	powdered CT1 diet, *ad libitum*	
Water:	*ad libitum*; not otherwise described	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	30-70%
	Air changes:	at least 15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Animals were supplied time-mated and arrived 6 days before dosing began	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: December 10, 2002; End: November 6, 2003.

2. **Study schedule:** Time-mated females were assigned to treatment groups upon arrival. The test substance was administered to the maternal animals from gestation day (GD) 7 through postnatal day (PND) 7, where the day of birth was designated as PND 1 or lactation day (LD) 1. Litter standardization and selection of F_1 pups were conducted on PND 5. The selected pups were dosed on PNDs 8 through 22 and remained on study until PND 63 (study termination). The selected pups were weaned on PND 29, at which time the maternal animals were killed and discarded.

3. **Mating procedure:** Females were naturally mated while at the supplier. The day on which spermatozoa were observed in a vaginal smear was designated as GD 1, and the females were shipped to the testing facility on this same day. It is unknown whether males of the same strain were used for mating.

4. **Animal Assignment:** Animal assignment is given in Table 1. Twenty time-mated females were supplied on each of 6 days and assigned to dose groups using a randomized block design to give a total of 30 replicates.

   Offspring were selected for use as F_1 animals at the time of litter standardization on PND 5. The offspring were allocated for use in neurobehavioral tests, brain weight determinations, and neuropathological evaluations by using one male pup *or* one female pup/litter in most cases; however, for some parameters one male pup *and* one female pup were selected from some high-dose litters due to the small number of available litters in this group. The functional observational battery, locomotor activity assessment, and PND 63 *post mortem* investigations used the first male or first female per litter; auditory startle habituation and PND 12 *post mortem* investigations used the third male or third female per litter; the second and fourth male and females of each litter were used in learning and memory.

Table 1. Study design.

Experimental Parameter	Dose (mg/kg bw/day)			
	0	0.1	1.0	7.5
Maternal Animals				
No. of maternal animals assigned	30	30	30	30
Offspring				
FOB (PNDs 5, 12, 22, 36, 46, and 61)	11-12/sex	10-11/sex	8-13/sex	8-10/sex
Motor activity (PNDs 14, 18, 22, and 60)	11-12/sex	7-11/sex	10/sex	7/sex
Auditory startle habituation (PNDs 23 and 61)	11/sex	10-11/sex	10/sex	5-7/sex
Learning and memory (PNDs 24-27 and 59-62)	21-23/sex	17-21/sex	18-21/sex	13/sex
Brain weight: PND 12 (fixed weight) PND 63 (wet weight)	 11/sex 11-12/sex	 10/sex 10-11/sex	 10-11/sex 10-11/sex	 6-7/sex 10-11/sex
Neuropathology and Morphometry: PND 12 (immersion fixation) PND 63 (perfusion fixation)	 11-12/sex 11-12/sex	 none none	 none none	 6-7/sex 10-11/sex

Data taken from text table, p. 19, and text, pp. 21-27, MRID 46153302.

5. **Dose selection rationale:** Dose levels were chosen based on the results of a preliminary developmental neurotoxicity study with Dichlorvos in the rat (MRID 46153302; see Appendix) in which gavage administration of the test material at 7.5 mg/kg bw/day to pregnant rats from GD 7 through PND 22 resulted in biologically significant decreases in

erythrocyte (RBC) and whole brain acetylcholinesterase (AChE) activities in maternal animals at GD 22 and at PND 22 and in fetuses of both sexes at GD 22. At 1.0 mg/kg bw/day, maternal animals had decreased RBC AChE activity at GD 22 and PND 22. Plasma AChE activity was not measured. The study author mentioned body weight decreases beginning on LD 11 in dams treated at 7.5 mg/kg bw/day, but these were of insufficient magnitude to be considered biologically significant (just 3-4% less than controls). According to the study report, in a repeat dose sensitivity study conducted at the same laboratory (Laboratory report number CTL/KR1490/Regulatory/Report), pre-weaning and young adult rats had decreased RBC and brain AchE activities at doses of 7.5 and 15 mg/kg bw/day. No further information about the repeat dose sensitivity study was available to the reviewer.

6. **Dosage administration:** All doses were administered once daily by gavage in de-ionized water at a dosing volume of 10 mL/kg bw/day, based on the most recent (daily) body weight determination. Maternal animals were dosed from GD 7 through PND 7, and F_1 animals were dosed on PNDs 8 through 22.

7. **Dosage preparation and analysis:**

The amount of the test material used was not adjusted to account for purity. Formulations were prepared every 4-6 days by adding sufficient de-ionized water to a weighed amount of test material to produce a high-dose stock solution, which was further diluted to attain mid- and low-dose formulations. Each batch of formulations was subdivided into aliquots for daily dosing and stored at room temperature until use. The method used to mix the formulations was not described, although the study report stated that the preparations were shaken prior to dose administration. Triplicate samples of low- and high-dose formulations from a pre-study batch (prepared on December 4, 2002) and from the first batch used in the study (prepared on December 12, 2002) were collected for stability analysis. Triplicate or duplicate samples of low-, mid-, and high-dose formulations from the first batch and from two subsequent batches (January 6, 2003; February 7 and/or 12, 2003) were analyzed for concentration. Homogeneity analysis was not done.

Results: Concentration Analysis: The study report stated that "re-analysis" was conducted on February 12 due to variability between the results from triplicate samples taken on February 7. However, it was unclear whether the initial samples were re-analyzed or whether additional samples were taken from a batch prepared on the later date; only the data from February 12 were reported. Absence of the test material was confirmed in the vehicle control formulations. Mean concentrations of the low-, mid-, and high-dose formulations were 116.0-125.0%, 105.0-112.0%, and 98.0-105.6% of nominal, respectively.

Stability Analysis: After 2, 5, and 8 days at room temperature, mean concentrations of the pre-study low-dose formulation were 93.3%, 108.6%, and 103.8% of initial, respectively, and the mean concentrations of the pre-study high-dose formulation were 92.6%, 90.3%, and 85.1% of initial, respectively. After 5 days at room temperature, the

mean concentrations of the low- and high-dose formulations from the first batch were 93.2% of initial and 94.2% of initial, respectively.

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 cage-side observations were conducted each morning and towards the end of each working day. Detailed clinical observations and body weights were recorded upon arrival, daily (immediately prior to dosing) during GD 7 through PND 7, and on PNDs 15, 22, and 28 (termination).

All maternal animals were subjected to a functional observational battery on GDs 10 and 17, and on PNDs 2 and 9. The examinations were conducted in the home cage and in a standard (open) arena by an individual who was unaware of each animal's treatment group and included evaluation of the parameters indicated (X) below. The testing procedure and scoring criteria were not described, but all observations were scored as "no abnormalities detected, slight, present, or left/right/bilateral."

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Lacrimation or salivation 2) Piloerection or endophthalmus/exophthalmus. 3) Urine staining or diarrhea 4) Pupillary response to light: miosis/mydriasis 5) Degree of palpebral closure, i.e. ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements in the home cage and standard (open) arena.
X	Reactivity to general stimuli, including response to approach and touch.
X	Arousal level/alertness.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behavior, excessive or repetitive action (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.

According to the study protocol, on treatment days the testing was done prior to dosing; however, this information was not included in the "experimental procedures" section of the study report. There was no description of the environmental conditions (e.g., noise level, etc.) during testing, and the study report did not specify the duration of the observation period in the open field or mention whether the same technicians were used throughout testing.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as PND or LD 1. The sex, weight, and clinical condition of each pup was recorded on PNDs 1 and 5, and litters were checked daily throughout lactation for dead, moribund, or "abnormal" pups.

On PND 5, litters were standardized to a maximum of 8 pups/litter (a randomly selected 4/sex/litter, as nearly as possible), and litters with 7-8 pups and at least 3 pups of each sex remained on study as the F_1 generation. The excess pups were killed and discarded.

The F_1 litters remained with their dams until PND 29. Individual body weight and detailed clinical observations were recorded on PND 5, daily during PNDs 8-22 (immediately prior to dosing), and on PND 29.

2) **Postweaning observations:** After weaning on postnatal day 29, offspring were examined daily for mortality or clinical signs. Individual body weights and detailed clinical observations were recorded on PNDs 36, 43, 50, 57, and 63 (prior to termination).

3) **Developmental landmarks:** Beginning on PND 29, female offspring were examined daily for vaginal patency, and beginning on PND 36, male offspring were examined daily for balanopreputial separation. The age and body weight at the time of onset were recorded for each animal.

4) **Neurobehavioral evaluations:**

a) **Functional observational battery (FOB):** Selected F_1 offspring were subjected to a functional observational battery on PNDs 5, 12, 22, 36, 46, and 61. The examinations were conducted in the home cage and in a standard (open) arena by an individual who was unaware of each animal's treatment group. On treatment days the testing was done prior to dosing. The FOB for offspring assessed the same parameters as the maternal FOB, and the observations were scored in the same manner as well, i.e. the severity scores included no abnormalities detected, slight, present, or left/right/bilateral. There was no adjustment of the FOB to account for developmental age. There was no description of the environmental conditions (e.g., noise level, etc.) during testing; the duration of the observation period for open field observations was not specified; and there was no mention of whether the same technicians were used throughout testing.

In general, one male *or* one female was selected from each litter; however, in order to ensure that at least 10 animals per sex were examined, it was necessary to select one male *and* one female from some high-dose litters. Many of the same offspring were evaluated at each time point. Some, but not all, of the instances when this did *not* occur appeared to be a later assignment of an additional animal as a substitute for one that had died. The other instances included the following: one low-dose female was evaluated only on PNDs 12 and 61; three mid-dose males were evaluated on PNDs 12, 22, 36, and 61, but not on PNDs 5 and 46; three mid-dose males were evaluated only on PND 46; one high-dose male and one high-dose female were evaluated only on PND 46; one high-dose female was evaluated on PNDs 12, 22, 36, 46, and 61 but not on PND 5; one high-dose female was only evaluated on PND 12;

and one high-dose female was evaluated on PNDs 12, 36, 46, and 61, but not on PND 5 or 22.

b) **Motor activity testing:** Motor activity was evaluated in one male *or* one female per litter on PNDs 14, 18, 22, and 60. An automated activity recording apparatus was used to record large and small movements over the course of a 50-minute session, comprised of ten 5-minute scans. The same animals were evaluated at each time point, except for one low-dose male that wasn't assigned until PND 18, and there was no replacement of animals that died between time points. On treatment days (PND 14, 18, and 22), the testing was done prior to dosing. The study report stated that the treatment groups were counterbalanced across the cage numbers of the activity monitors and that the assessments were done in a separate room in order to minimize environmental distraction. During each session (or run), up to 16 cages were monitored, and each animal was tested in the same monitoring device across test sessions. Replicates were not used because there were enough devices available to conduct a single run on each date when the testing was done. A description (or make and model number) of the monitoring devices was not provided.

c) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on one male *or* one female per litter on PNDs 23 and 61, using an automated system. On each day of testing, there were two sessions comprised of 5 blocks of 10 trials. Up to 8 animals were evaluated during each session, and each animal was tested in the same chamber and at the same time (session 1 or 2) across testing days. The study report did not state whether treatment groups were counterbalanced across chamber numbers and session times, but this appeared to be the case. There was no description of the equipment used, environmental conditions, length (msec) and intensity (dB) of sound, or the length of the interval between trials.

d) **Learning and memory testing:** Water maze testing was performed on PNDs 24-27 and on PNDs 59-62 to evaluate associative learning and memory. Separate groups of one animal/sex/litter were tested at each interval, and each animal was tested twice, with 2 days between test sessions. Testing equipment included a straight channel "maze,"and a Y-shaped maze with one escape ladder. Each session was comprised of 6 trials in the Y-shaped maze followed by a single trial in the straight channel to evaluate swim speed. The amount of time required for the animal to find the ladder was recorded for each trial.

The criterion for a successful trial was a time less than a given cut-off value, and the following cut-off values were used: 3, 4, 5, 6, 7, 8, 9, and 10 seconds; and multiples of 1.0, 1.5, and 2.0 times the individual animal's straight-channel time. For each individual, the percentage of trials meeting a specific criterion was calculated and used to determine the group mean for that criterion.

Learning was assessed by comparing the swim times for Trials 1 and 6 on the first day of testing, and memory was assessed by comparing the swim time for Trial 1 on the second day of testing to the swim time for Trial 1 on the first day.

The inter-trial interval was not reported and there was no further description of the equipment or environmental conditions (lighting, water temperature and depth, background noise, etc.).

5) **Cholinesterase determination:** Biomarker data were not collected from offspring in the main study.

2. **Postmortem observations:**

a. **Maternal animals:** Females that failed to litter were sacrificed on nominal GD 25 by halothane vapor overdose with subsequent exsanguination and subjected to a gross necropsy which included examination for pregnancy status. Animals showing signs of moribundity and/or dystocia and some (but not all) of the females with total litter losses were sacrificed and examined in the same manner as the females that failed to litter; animals that were found dead were also subjected to gross necropsy. Dams with litters not selected as F_1 animals on PND 5 and most of the females with total litter losses were sacrificed by an unspecified method and discarded without examination. Maternal animals of the selected F_1 litters were sacrificed by carbon dioxide asphyxiation on PND 29 and discarded without examination. No tissues were retained or processed for histopathological examination.

b. **Offspring:** On PND 5, the excess pups (i.e. those culled during litter standardization and litters not selected as F_1 animals) were killed by an unspecified method and discarded without examination. Offspring that were found dead during the dosing interval (PND 8-22) were subjected to gross necropsy. Offspring that died or were killed for humane reasons prior to PND 8 or after PND 22 generally were discarded without examination, but "a small number" of the offspring that died were examined to determine a cause of death. No tissues were retained from these animals.

The offspring selected for brain weight and/or neuropathological evaluation were sacrificed on PND 12 or on PND 63 and subjected to postmortem examinations as described below.

On postnatal day 12, 6-11 pups/sex/group (one male or one female per litter) were sacrificed by carbon dioxide exposure, and the brains from these animals were immediately exposed and immersion fixed in 10% neutral buffered formol for at least 24 hours. Fixed whole brain and cerebellar weight were recorded, and brain from the control and high-dose pups was processed in the following manner. The cerebellum was cut sagitally at midline to make 2 blocks (20 and 21) and the remainder of the brain was cut into 5 blocks by making transverse cuts at the following anatomic landmarks: the rostral edge of the olfactory bulb (level 1); the caudal edge of the olfactory bulb (level 2); the rostral edge of the median eminence (level 3); the caudal edge of the cerebral hemispheres (level 6); and the midpoint of the remaining brain stem. The blocks were embedded in paraffin with the rostral or medial face down (as appropriate), sectioned, stained with hematoxylin and eosin, and examined using light microscropy.

An image analysis system (KS400) was used to make the morphometric measurements given in Table 2. The system used a light box, macro lens, and video camera, calibrated by means of a graticule, to take the measurements on levels 2-5 of the cerebrum/brainstem and to measure the height and length of the section of the cerebellum. The rest of the cerebellum measurements were made using a light microscope, calibrated by means of a stage micrometer. Measurements of width, length, and height were made over the maximum dimension of the indicated structure, and dorsal cortex measurements were made at right angles to a tangential line at the surface of the brain and extended from the meningeal surface

to the inner edge of the pyramidal cells adjacent to the white matter of the external capsule. Bilateral features on the cerebrum/brainstem sections were measured on both the left and right sides unless one side was oblique or failed to show the feature in question for some other reason. The cerebellum was measured on one of the two slides, i.e. the one that provided the best sagittal section. In some cases, it was not possible to cut an adequate section for one of the levels.

The image analysis system was also used to measure the length of the Purkinje cell layer on lobule 8 of the cerebellum adjacent to the prepyramidal fissure. The number of Purkinje cell bodies in lobule 8 were counted and expressed as a function of the length of lobule 8.

Table 2. Brain morphometry.		
Brain Region	**Parameter Description and [Number]**	
Frontal Cortex	Height	[2A]
	Width	[2B]
Dorsal Cortex	Thickness on Level 3 at most dorsal point of external capsule, parallel to midsagittal line	[3A]
	Thickness on Level 3 along a line drawn at ~45°from the midsagittal plane	[3B]
	Thickness on Level 4 along a line drawn at 90°to the surface and through the medial tip of the dentate gyrus	[4A]
	Thickness on Level 5, measured in the same manner as 4A (immediately above)	[5A]
Piriform Cortex	Thickness on Level 3 at midpoint between rhinal and amygdaloid fissures	[3C]
	Thickness on Level 4 at midpoint between rhinal and amygdaloid fissures	[4B]
	Thickness on Level 5 at midpoint between rhinal and amygdaloid fissures	[5B]
Hippocampus	Length from midline to outer edge of most lateral pyramidal cells on Level 3	[3D]
	Length from midline to outer edge of most lateral pyramidal cells on Level 4	[4G]
	Width on Level 5 from inner zone of dentate gyrus to outer edge of CA2 [a]	[5E]
	Dentate gyrus: Width on Level 4 at level of most medial part of lower limb of CA3 [a]	[4H]
	Length on Level 4, measured parallel to a dorsal (horizontal) plane	[4J]
	Width at widest point on Level 5	[5D]
Corpus Callosum	Thickness at midline on Level 4	[4C]
Thalamus	Height at midline on Level 4	[4D]
	Width at widest point on Level 4	[4E]
	Width at widest point on Level 5	[5C]
Thalamus/Cortex	Overall width at the widest point of Level 4	[4F]
Cerebellum	Height	[8H]
	Length	[8L]
	Preculminate Fissure: Thickness of molecular layer	[8PCFM]
	Thickness of outer granular layer [b]	[8PCFO]
	Thickness of inner granular layer	[8PCFI]
	Prepyramidal Fissure: Thickness of molecular layer	[8PPFM]
	Thickness of outer granular layer [b]	[8PPFO]
	Thickness of inner granular layer	[8PPFI]

Data taken from Appendix F, pp. 220-225, MRID 46153302.
[a] CA2 = Cornu Ammonis 2, and CA3 = Cornu Ammonis 3.
[b] Measured only in pups killed on PND 12; not found in adult rats.

On postnatal day 63, at least 10 animals/sex/group were deeply anesthetized via intraperitoneal sodium pentobarbitone and euthanized by perfusion fixation with a volume of formol saline approximately equivalent to the animal's body weight. Brains were immediately removed, whole brain and cerebellar weights were recorded, and the central and peripheral nervous tissues indicated below (X) were collected and preserved in an unspecified "appropriate" fixative. The tissues from the control and high-dose animals were processed in the following manner and examined. The cerebellum was cut sagittally at midline to make 2 blocks (levels 20 and 21), and the remainder of the brain (cerebrum and brain stem) was cut into 6 blocks by making transverse cuts at the following anatomic landmarks: the rostral edge of the olfactory bulb (level 1); the caudal edge of the olfactory bulb (level 2); the rostral edge of the median eminence (level 3); the caudal edge of the median eminence (level 5); the caudal edge of the cerebral hemispheres (level 6); and the midpoint of the remaining brain stem. The blocks were embedded in paraffin with the rostral or medial face down (as appropriate), sectioned, and stained with hematoxylin and eosin, and the spinal cord sections (including spinal nerve roots and dorsal root ganglia), eyes, and muscle sections were processed in the same manner. The peripheral nerve tissues were embedded in resin, sectioned in a "semi-thin" manner, and stained with toluidine blue. Detailed morphometric evaluations and enumeration of Purkinje cell bodies in lobule 8 of the cerebellum were conducted in the same manner as for pups killed on PND 12.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES** [transverse and longitudinal sections]
X	Cerebrum and brainstem (transverse sections)	X	Proximal sciatic nerve [a]
X	Cerebellum (sagittal sections)	X	Proximal tibial nerve [a]
		X	Distal tibial nerve (calf muscle branches) [a]
	SPINAL CORD [transverse and longitudinal sections]		**OTHER**
X	Cervical swelling	X	Eye (with optic nerve and retina) [a]
X	Lumbar swelling	X	Gastrocnemius muscle (transverse sections) [a]
		X	Spinal nerve roots at cervical swelling [b]
		X	Spinal nerve roots at lumbar swelling [b]
		X	Dorsal root ganglia at cervical swelling [b]
		X	Dorsal root ganglia at lumbar swelling [b]

Data taken from pp. 26-27, MRID 46153302.
[a] Right and left preserved; left processed for examination.
[b] Spinal nerve roots and dorsal root ganglia were included in transverse sections of the spinal cord.

In addition, at least 10 animals/sex/group were sacrificed on PND 63 by carbon dioxide exposure, and the brains from these animals were immediately removed, weighed (whole brain and removed cerebellum), and stored in an unspecified fixative.

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No qualitative or quantitative evaluation of brain from pups or adult offspring of the low- and mid-dose groups was conducted.

D. DATA ANALYSIS:

1. **Statistical analyses:** Maternal body weight during gestation and during lactation were analyzed using analysis of covariance (ANCOVA) with GD 7 body weight and LD 1 body weight, respectively as covariants. Maternal body weight on LD 1 was analyzed using an analysis of variance (ANOVA), and maternal body weight on GDs 1 and 7 apparently was not analyzed statistically.

   Offspring body weight was evaluated on a litter basis. ANCOVA was used to analyze the mean pup weight on PND 5 pre-cull and to analyze the mean weight of the selected F_1 offspring during PNDs 8-63. The mean body weight on PND 1 and on PND 5 post cull were respectively used as covariants, and both were analyzed used ANOVA.

   The following data were analyzed using ANOVA: gestation length; litter size; total litter weight on PNDs 1 and 5; motor activity measurements; max amplitude and time to maximum amplitude in startle response tests; (litter based) time to preputial separation or vaginal opening; (litter based) body weight at preputial separation or vaginal opening; brain morphometry data; and the number of Purkinje cell bodies per mm.

   Whole brain and cerebellum weights were analyzed using ANOVA and using ANCOVA with final body weight as the covariate. Brain to body weight ratio was not analyzed statistically.

   The following parameters were analyzed using Fisher's Exact Test: the proportion of litters with gestation length less than, equal to, and greater than 22 days; the proportion of whole litter loss in each group; and the proportion of males and females with observed developmental landmarks (preputial separation and vaginal opening) on each day.

   Data pertaining to live born pups, pup survival pre- and post-cull, and pup sex were evaluated as follows: 1) mean percentages were analyzed using ANOVA following the double arcsine transformation of Freeman and Tukey; 2) the proportion of pups born alive, the proportion of pups surviving, the proportion of litters with all pups born alive, the proportion of litters with all pups surviving and the proportion of male pups were analyzed using Fisher's Exact Test.

   Data from the water maze testing were analyzed as follows: 1) mean swimming times in the straight channel and for each individual trial in the Y-maze were analyzed using ANOVA; 2) mean percentages of successful trials at each cut-off value were analyzed using ANOVA following the double arcsine transformation of Freeman and Tukey.

   All statistical tests were two-sided and used significance levels of $p<0.05$ and $p<0.01$.

2. **Indices:**

a. **Reproductive indices:** No reproductive indices were calculated.

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

Live Birth Index (%) = (Number of pups born alive/Total number of pups born)×100.

Viability Index (%) = (Number of pups alive on LD 5/Number of pups born alive)×100, calculated both including and excluding litters with total litter losses.

3. **Positive and historical control data:** Historical control data were provided for the incidences of minimal and slight demyelination of the proximal sciatic, proximal tibial, and distal tibial nerves. The data came from 4 studies conducted during October 2001 through July 2002. No further information was provided concerning the materials, methods, and personnel used in those studies.

No positive control data were provided. However, the following citations for previously conducted positive control and/or methodology validation studies were included in the "References" section of the study report (p. 36, MRID 46153302):

- Allen, S. (1993) Measurement of motor activity in rat pups. CTL Report No. CTL/P/4155. MRID 44064701.
- Allen, S. (1994) Assessment of learning and memory in rats. CTL Report No. CTL/P/4257. MRID 44064702.
- Allen, S. (1996) Trimethyltin chloride: investigation of neurotoxicity in rat pups using morphometrics and startle response. MRID 44064703.
- Allen, S. (1995) Developmental neurotoxicity study in the rat using dietary restriction. CTL Report No. CTL/P/4383. MRID 44064705.
- Chivers, S. (2003) Motor activity: positive control study in rat pups. CTL Report No. CTL/WR0475/Regulatory/Report.
- Milburn, G. (2003) Dizocilpine and mecamylamine: positive control water maze study in rats. CTL Report No. CTL/WR0442/Regulatory/Report.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** One high-dose female was sacrificed on LD 3 due to clinical signs of discharge from the left eye, pallor, piloerection, and slightly hunched posture and thin appearance. One mid-dose female died on GD 24 due to parturition difficulties.

   No abnormal FOB findings were recorded on GD 10, GD 17, or LD 9. The following abnormal FOB findings were recorded on LD 2: chromodacryorrhea (graded as "bilateral") in one high-dose female; paleness (graded as "present") in one mid-dose female; piloerection (graded as "slight") in one low-dose and one mid-dose female; and thinness (graded as "slight") in 2 low-dose females. All of these observations were of 1-3 days duration and resolved by LD 4. These findings were not considered treatment-related because each was only present in one or two animals, and there was no evidence of a dose response.

2. **Body weight:** Selected group mean body weight data for pregnant or nursing dams are given in Table 3. Mean body weight and body weight gain of the treated dams were similar to those of controls throughout gestation and lactation.

Table 3. Maternal body weight [a]

Observations/study day	Dose (mg/kg bw/day)			
	Control	0.1	1.0	7.5
	Gestation	**[N = 30, 30, 29, and 27 dams]**		
Mean body weight (g):				
GD1	256.9±15.8	257.1±18.4	257.4±18.4	257.9±16.8
GD 7	292.6±14.2	291.5±17.5	289.0±15.8	292.5±18.4
GD 14	330.7±15.2	330.5±19.7	325.5±15.7	328.6±20.3
GD 22	395.9±22.4	394.1±25.0	393.0±21.3	402.0±28.9
Weight gain (g) [b]:				
GD 1-7	35.7	34.4	31.6	34.6
GD 7-14	38.1	39	36.5	36.1
GD 14-22	65.2	63.6	67.5	73.4
	Lactation			
Mean body weight (g):				
LD 1 [N = 24, 27, 22, 21]	299.5±22.6	298.3±33.0	299.2±19.4	309.4±30.9
LD 7 [N = 23, 21, 20, 13]	313.7±20.3	310.0±21.8	312.3±19.6	321.8±22.9
LD 15 [N = 23, 21, 20, 13]	348.3±21.5	343.5±19.7	347.2±16.4	363.3±24.7
LD 22 [N = 23, 21, 20, 13]	355.7±16.0	354.0±17.3	353.6±16.8	367.5±14.6
LD 29 [N = 23, 20, 20, 13]	345.3±16.5	341.1±19.7	337.1±21.5	347.5±12.6
Weight gain (g) [b]:				
LD 1-7	14.2	11.7	13.1	12.4
LD 7-15	34.6	33.5	34.9	41.5
LD 15-22	7.4	10.5	6.4	4.2
LD 22-29	-10.4	-12.9	-16.5	-20

Data taken from Tables 4 and 5, pp. 72-74 and 75-76, respectively, MRID 46153302.
[a] Mean body weight values are given as Mean ± Standard Deviation, with group sizes as indicated.
[b] Calculated by reviewer using group mean body weight values; not analyzed statistically.

3. **Reproductive performance:** The reproductive performance of the F_0 females is summarized in Table 4. There was no treatment-related effect on gestation length. Two low-dose dams littered on GD 23, one mid-dose female was found dead due to dystocia on GD 24, and the remaining dams delivered on GD 22. One mid-dose female had a litter of 14 dead pups (and none live), and two high-dose females had total litter resorptions.

Table 4. Reproductive performance.				
Observation	Dose (mg/kg bw/day)			
	Control	0.1	1.0	7.5
Number mated	30	30	30	30
Number pregnant (%)	30 (100%)	30 (100%)	29 (96.7%)	29 (96.7%)
Incidence of dystocia	0	0	1	0
Total litter resorptions	0	0	0	2
Litters born dead	0	0	1	0
Number of litters with live pups on LD 1	30	30	27	27
Intercurrent death or moribund sacrifice	0	0	0	1
Mean (±SD) gestation duration (days)	22.0±0.0	22.1±0.3	22.0±0.0	22.0±0.0

Data taken from text and text table, p. 29, Table 6 (p. 77), Appendix 4 (p. 772), and Appendix 5 (pp. 776-786), MRID 46153302.

4. **Maternal postmortem results:** Ten F_0 females were necropsied. These included the mid-dose female that died from dystocia, the high-dose female that was sacrificed moribund, four females that failed to litter by GD 25 (1 mid- and 3 high-dose females), and four dams with total litter losses (2, 1, and 1 from the low-, mid-, and high-dose groups, respectively). A pale liver was noted in the female that was sacrificed moribund. The female that died due to dystocia had one dead fetus in the vagina and other dead fetuses in the uterine horns, with no abnormalities detected in other tissues. Recorded observations from the 8 remaining animals were limited to counts of implantation sites and/or dead fetuses in uterine horns or a statement that implantation sites were absent. The results from one animal included a statement that no abnormalities were detected in other tissues, and it is unclear whether any other organs or tissues were examined in the remaining 7 animals.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) are summarized in Table 5. In all groups, including controls, there were inordinate numbers of pup deaths and total litter losses between LD 1-5; these findings were considered incidental to treatment. During LD 5-22, the number of pups found dead (with or without cannibalization) or missing/presumed dead in all treated and control groups remained high. Other clinical observations included such findings as hypothermia, pale pups, damaged tails, and injured limbs, and none appeared dose- or treatment-related.

Table 5. Litter size and viability

Observation		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
Total number born		368	371	354	340
Number born alive		363	367	331	330
Number born dead		5	4	23	10
Number alive LD 5, pre-cull		281	303	273	228
Number alive LD 5, post-cull [a]:	F_1 Males:	91	83	83	56
	F_1 Females:	93	84	84	56
	Total F_1 animals:	184	167	167	112
Deaths [Number of pups] LD 1-5: [a,b]		82	64	58	89
Deaths [Number of F_1 offspring] LD 5-22: [a]					
	Males:	0	7	7	11
	Females:	9	5	4	2
	Combined:	9	12	11	13
Mean litter size [c]:	LD 1:	12.1±2.3	12.0±2.6	12.0±2.2	12.1±3.1
	LD 5 (pre-cull)	11.7±2.6	11.2±3.1	11.9±2.2	10.9±3.8
Sex Ratio (% male):	LD 1:	48.8	47.4	45.6	49.7
	LD 5:	49.8	49.2	46.2	50.4
Live birth index (%) [d]		98.6	98.9	93.5	97.1
Viability index (%) [e]		77.4	82.6	82.5	69.0
Litter disposition: Number with live pups on LD 1		30	30	27	27
Total Litter Losses LD 1-5		6	3	5 [f]	5
Number lost to maternal sacrifice		0	0	0	1
Number used as F_1 offspring		23	21	21	14
Number killed LD 5 [unsuitable as F_1]		1	6	2	7

Data taken fro m Tables 7, 9, 10, 11, 14, and 15, pp. 78, 80, 81-82, 83, 86, and 87-93, respectively, MRID 46153302.

[a] Calculated by reviewer.

[b] Excludes data from litter of high-dose dam sacrificed moribund.

[c] Values given as Mean ± Standard Deviation, with N = 24, 27, 23, and 22 on LD 1, and N = 24, 27, 23, and 21 on LD 5; data excluded from litters with total litter losses.

[d] Live Birth Index (%) = (Number of pups born alive/Total number of pups born)×100; calculated by reviewer.

[e] Viability Index (%) = (Number of pups alive on LD 5/Number of pups born alive)×100; calculated by reviewer.

[f] Includes one mid-dose litter that was born dead.

2. **Body weight:** Pre- and post-weaning offspring body weight data are summarized in Tables 6 and 7. There were no adverse treatment-related effects on offspring body weight during or

after lactation. Small but statistically significant increases in body weight of high-dose males and females were not considered toxicologically relevant.

Table 6. Pre-weaning offspring body weight data (g) [a]

Parameter/Postnatal Day or Interval		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
Males					
Mean body weight: PND 1	[N=30, 30, 27, 27]	5.9±0.6	6.0±0.7	5.9±0.6	6.1±0.8
PND 5 (pre-cull)	[N=24, 27, 23, 21]	9.5±1.3	9.4±1.5	9.5±1.2	9.9±1.5
PND 5 (post-cull)	[N=23, 21, 21, 14]	9.4±1.1	9.4±1.2	9.5±1.1	9.8±1.1
PND 12		22.6±2.1	22.5±2.1	23.7±2.6	24.2±2.7
PND 17	Unadjusted and [Adjusted] [b]	35.1±2.8 [35.3]	35.4±3.4 [35.6]	36.8±3.5 [36.7]	38.0±3.9 [37.6 * (107) [c]]
PND 22	Unadjusted and [Adjusted]	51.2±3.8 [51.4]	51.6±3.8 [51.8]	53.2±4.1 [53.1]	55.4±4.8 [54.9 ** (107)]
BW Gain [d]:	PND 1-5 (pre-cull)	3.6	3.4	3.6	3.8
	PND 5 (post-cull) through PND 22	41.8	42.2	43.7	45.6 (109)
Females					
Mean body weight: PND 1	[N=30, 30, 27, 26]	5.5±0.6	5.7±0.6	5.6±0.6	5.6±0.6
PND 5 (pre-cull)	[N=24, 27, 23, 19]	9.1±1.3	9.0±1.5	9.2±1.2	9.3±1.2
PND 5 (post-cull)	[N=23, 21, 21, 14]	9.0±1.2	8.8±1.2	9.2±1.1	9.3±1.1
PND 12		21.9±2.3	21.5±2.1	22.9±2.5	23.5±2.2
PND 17	Unadjusted and [Adjusted]	34.4±3.0 [34.4]	33.9±2.7 [34.2]	35.5±3.0 [35.4]	36.8±2.9 [36.5 * (106)]
PND 22	Unadjusted and [Adjusted]	49.7±3.9 [49.7]	49.7±3.6 [50.1]	51.5±3.4 [51.3]	53.3±3.4 [52.9 ** (106)]
BW Gain [d]:	PND 1-5 (pre-cull)	3.6	3.3	3.6	3.7
	PND 5 (post-cull) through PND 22	40.7	40.9	42.3	44.0

Data taken from Tables 12 and 16, pp. 84 and 124-129, respectively, MRID 46153302.

[a] Values are given as Mean ± Standard Deviation, calculated on a litter basis. Group sizes [N] are indicated each time there is a change in number.

[b] Data were analyzed using ANCOVA, with post-cull PND 5 body weight as the covariate. Covariate-adjusted means are provided when statistical significance was found.

[c] Numbers in parentheses equal percent of control; calculated by reviewer.

[d] Calculated by reviewer using group mean body weight values; not analyzed statistically.

Significantly different from control: * $p<0.05$; ** $p<0.01$.

Table 7. Post-weaning offspring body weight data (g) [a]				
Parameter/Postnatal Day or Interval	**Dose (mg/kg bw/day)**			
	Control	**0.1**	**1.0**	**7.5**
Males				
Mean body weight:				
PND 29 Unadjusted and [Adjusted] [b]	91.2±6.2 [91.5]	92.0±5.1 [92.3]	92.9±7.0 [92.8]	96.3±7.1 [95.5 * (104) [c]]
PND 36 Unadjusted and [Adjusted]	143.4±9.5 [143.8]	141.9±7.9 [142.3]	146.8±9.6 [146.6]	151.5±11.8 [150.3 * (105)]
PND 50	253.5±16.7	253.4±13.6	259.4±16.3	265.7±17.6
PND 63	344.2±22.1	344.2±18.2	352.7±24.1	359.6±23.00
BW Gain [d]: PND 29-63	253.0	252.2	259.8	263.3
Females				
Mean body weight:				
PND 29	85.6±6.2	85.6±4.8	87.3±6.2	89.7±4.7
PND 36 Unadjusted and [Adjusted]	125.9±7.5 [126.1]	125.8±7.2 [126.5]	130.2±7.5 [129.8]	132.1±7.7 [131.3 * (104)]
PND 50	181.4±12.5	185.1±9.9	187.4±11.6	187.7±7.8
PND 63	210.7±17.2	215.9±15.7	219.8±13.6	219.8±7.3
BW Gain [d]: PND 29-63	125.1	130.3	132.5	130.1

Data taken from Table 16, pp. 124-129, respectively, MRID 46153302.
[a] Values are given as Mean ± Standard Deviation; calculated on a litter basis with N=23, 21, 21, and 14 for control, low-, mid-, and high-dose groups, respectively.
[b] Data were analyzed using ANCOVA, with post-cull PND 5 body weight as the covariate. Covariate-adjusted means are provided when statistical significance was found.
[c] Numbers in parentheses equal percent of control; calculated by reviewer.
[d] Calculated by reviewer using group mean body weight values; not analyzed statistically.
Significantly different from control: * $p<0.05$, ** $p<0.01$.

3. Developmental landmarks:

a) Sexual maturation: Data pertaining to offspring sexual maturation are reported in Table 8. There were no biologically relevant effects.

Table 8. Mean age and body weight at sexual maturation [a]				
Parameter	Dose (mg/kg bw/day)			
	Control	0.1	1.0	7.5
N (M/F)	23/23	21/21	21/21	14/14
Preputial separation (males)				
Age (days)	44.6±1.4	44.4±1.1	43.9±1.2	43.8±1.2
Body weight (g)	208.7±12.3	207.3±10.6	208.0±14.0	213.2±11.6
Vaginal opening (females)				
Age (days)	36.8±1.4	37.2±1.3	37.0±1.3	37.2±2.2
Body weight (g)	129.6±11.3	131.1±9.4	134.6±10.0	137.8±12.9 * (106)

Data taken from Table 17, pp. 130-131, MRID 46153302.
[a] Values are given as Mean ± Standard Deviation, with group sizes [N] as indicated.
Significantly different from control: $p<0.05$; $p<0.01$.

4. Behavioral assessments:

a) **Functional observational battery:** No treatment-related findings were seen during FOB testing of the F_0 animals. Exophthalmos was noted (unilaterally) in 1/13 mid-dose females at PND 46. All other FOB observations at all other time points were scored as "no abnormalities detected."

b) **Motor activity:** The motor activity data are reported in Tables 9 (total activity counts) and 10a and 10b (sub-session data from males and females, respectively). Total activity counts generally increased with increasing age, and no significant differences were found between the total activity counts of the treated and control groups of either sex on any testing day. Statistically significant differences were noted sporadically for individual sub-sessions, but no dose- or time-related pattern was evident. It should be noted that habituation was not evident for any group on any day, including controls, and the sub-session counts were highly variable between successive intervals within sessions.

Table 9. Motor activity data: total activity counts for session [a]

Test Day	Dose (mg/kg bw/day)			
	Control	0.1	1.0	7.5
		Males		
PND 14 [N=12, 10, 10, 7]	192.3±126.7	267.2±189.7	160.1±146.5	194.7±169.0
PND 18 [N=12, 11, 10, 5]	241.8±129.1	192.2±125.4	135.7±111.6	241.2±228.3
PND 22 [N=12, 11, 9, 5]	435.9±144.3	384.5±164.3	363.8±152.4	386.6±149.1
PND 60 [n=12, 11, 9, 5]	430.4±117.2	488.8±154.7	467.8±119.3	391.0±123.5
		Females		
PND 14 [N=11, 7, 10, 7]	223.3±110.9	166.0±181.1	256.8±166.3	213.6±190.1
PND 18 [N=11, 7, 10, 7]	237.7±146.3	199.9±172.3	194.7±91.2	201.3±105.3
PND 22 [N=11, 7, 10, 7]	310.1±144.8	326.0±100.6	341.0±138.3	386.6±88.8
PND 60 [N=11, 7, 10, 7]	558.4±121.5	577.7±109.8	641.2±80.7	578.7±92.6

Data taken from Table 18, pp. 132-, MRID 46153302.

[a] Values given as Mean ± Standard Deviation, with group sizes [N] as indicated.

Table 10a. Motor activity sub-session data from males (activity counts/sub-session) [a]					
Test Day/Sub-session		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
PND 14	1	37.4±28.0	41.8±21.9	22.7±22.6	28.9±21.0
[N=12, 10, 10, 7]	2	24.5±23.1	37.6±22.1	28.1±23.4	26.7±27.1
	3	21.2±17.3	34.9±21.2	19.1±18.6	21.4±21.9
	4	24.7±21.0	29.0±19.2	25.1±23.4	32.6±26.2
	5	23.2±18.3	35.5±29.0	15.0±17.5	11.9±10.1
	6	12.5±12.3	22.7±23.1	7.4±14.5	10.9±12.4
	7	16.6±26.0	11.0±16.3	14.8±20.8	19.9±26.7
	8	9.3±13.0	24.2±37.2	13.6±19.5	21.0±28.5
	9	12.2±13.3	18.4±26.0	7.4±12.2	14.9±16.5
	10	10.9±15.1	12.1±22.5	6.9±12.9	6.7±9.6
PND 18	1	27.7±16.4	26.5±21.3	15.2±14.8	26.2±28.6
[N=12, 11, 10, 5]	2	31.7±16.4	30.6±20.6	15.1±11.3 *	36.4±21.5
	3	26.3±21.0	19.4±20.1	15.3±17.3	34.2±28.1
	4	21.7±21.7	27.5±29.8	13.9±14.7	19.8±30.5
	5	22.6±31.4	19.4±19.5	20.3±26.7	17.8±25.4
	6	29.4±31.5	17.6±24.2	12.6±23.7	19.2±23.7
	7	23.4±25.6	12.8±17.4	12.1±24.8	19.8±27.4
	8	15.6±23.3	14.7±15.9	7.9±13.8	20.4±27.9
	9	21.8±27.3	10.2±16.7	14.5±21.2	24.2±31.1
	10	21.6±28.8	13.5±21.8	8.8±13.8	23.2±34.5
PND 22	1	45.5±22.0	36.0±27.1	41.9±17.0	47.0±12.5
[N=12, 11, 9, 5]	2	43.7±16.9	33.8±24.2	35.3±19.6	36.8±26.2
	3	41.8±27.0	29.4±24.8	31.9±19.6	40.8±17.3
	4	47.3±32.5	40.3±28.1	53.1±13.8	29.8±27.5
	5	40.2±28.2	39.6±26.3	40.3±18.3	54.2±11.0
	6	52.5±21.0	39.5±14.8	27.8±24.7 *	26.0±27.0 *
	7	46.0±19.4	48.9±20.9	26.0±25.5	38.0±27.2
	8	41.8±27.3	44.5±29.0	29.7±28.3	41.0±23.2
	9	41.2±27.2	38.2±28.1	38.3±25.4	30.2±21.9
	10	36.1±26.0	34.2±25.4	39.4±27.9	42.8±30.0
PND 60	1	67.2±7.9	67.9±9.6	66.1±7.3	58.0±8.1 *
[N=12, 11, 9, 5]	2	64.2±8.1	62.7±18.5	61.4±13.2	53.6±11.6
	3	58.7±20.6	51.7±22.0	59.0±13.4	52.0±12.3
	4	47.8±22.1	55.8±17.4	54.0±12.1	40.8±21.4
	5	34.1±20.2	37.9±31.9	38.0±27.5	40.6±21.7
	6	24.2±24.0	42.3±26.9	27.3±27.6	51.6±29.2
	7	36.3±27.5	39.9±29.6	37.6±28.4	29.4±19.7
	8	34.0±28.3	45.6±30.5	38.7±30.6	21.0±25.4
	9	38.6±29.7	49.2±24.4	41.7±28.7	17.2±25.6
	10	25.5±23.5	41.7±29.3	44.0±26.4	26.8±21.5

Data taken from Table 18, pp. 132-139, MRID 46153302.

[a] Values are given as Mean ± Standard Deviation, with group size [N] as indicated.
Significantly different from control: * $p<0.05$; ** $p<0.01$

Table 10b. Motor activity sub-session data from females (activity counts/subsession) [a]					
Test Day/Sub-session		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
PND 14 [N=11, 7, 10, 7]	1	36.8±20.3	25.6±26.8	27.8±24.2	43.0±29.5
	2	27.0±20.0	19.9±25.9	30.7±19.0	29.3±30.0
	3	29.7±18.7	25.1±21.8	31.4±22.4	31.1±28.5
	4	25.3±20.0	21.4±23.8	38.3±26.1	15.4±13.5
	5	12.4±13.2	13.7±23.9	28.2±30.2	19.1±23.5
	6	18.2±19.5	9.9±17.3	23.6±27.5	19.3±18.4
	7	21.3±17.4	10.9±17.7	25.7±21.0	13.9±13.9
	8	20.6±17.2	16.3±21.5	16.9±16.2	9.1±16.6
	9	15.7±14.7	9.6±16.0	21.2±24.6	13.1±17.6
	10	16.3±15.7	13.7±17.8	13.0±18.1	20.1±31.7
PND 18 [N=11, 7, 10, 7]	1	25.6±22.0	27.7±17.1	26.0±23.0	14.0±15.3
	2	30.4±28.9	28.6±24.5	15.7±16.1	21.9±19.8
	3	33.5±22.9	25.3±16.9	28.9±32.4	27.6±26.7
	4	26.6±27.4	23.9±24.3	15.9±23.5	13.7±17.4
	5	18.7±14.8	26.9±26.6	26.6±22.3	11.3±14.7
	6	16.7±18.4	8.7±22.6	23.3±20.2	18.6±22.1
	7	23.1±25.5	15.7±20.9	19.5±18.1	24.7±25.6
	8	22.9±22.5	14.4±24.8	17.4±25.1	14.0±17.2
	9	18.8±22.9	14.4±25.5	11.6±18.4	26.6±24.8
	10	21.3±21.9	14.3±22.6	9.8±20.1	29.0±30.1
PND 22 [N=11, 7, 10, 7]	1	26.5±22.4	21.9±16.2	36.9±27.0	36.3±13.3
	2	24.3±20.2	22.4±15.0	29.4±18.8	35.3±22.7
	3	33.5±22.0	23.1±15.6	25.4±22.5	42.4±17.0
	4	25.7±23.3	32.6±17.4	39.1±27.5	40.3±24.3
	5	22.2±25.1	26.3±17.0	38.6±29.3	28.6±22.9
	6	24.9±19.1	28.9±22.8	37.6±26.0	45.0±22.7
	7	41.0±26.3	42.7±14.2	31.2±24.4	33.4±23.1
	8	41.9±20.3	39.9±14.6	39.5±22.3	50.4±30.2
	9	42.5±26.0	41.0±19.7	33.9±25.5	29.7±22.7
	10	27.6±24.0	47.3±13.8	29.4±23.3	45.1±32.4
PND 60 [N=11, 7, 10, 7]	1	62.3±9.1	59.3±10.1	66.7±8.6	62.6±17.2
	2	61.3±13.7	64.1±10.4	61.4±14.1	61.4±17.0
	3	61.4±19.1	57.6±14.1	67.6±8.0	64.7±15.3
	4	58.1±17.8	64.0±15.1	64.9±12.9	64.6±14.8
	5	58.7±11.4	63.1±17.8	71.5±7.0 *	64.1±13.5
	6	48.2±10.9	53.3±20.7	63.0±11.1	56.4±28.3
	7	52.6±22.7	53.7±11.0	64.5±16.7	45.4±24.5
	8	52.0±24.2	49.0±11.2	55.4±23.1	43.1±26.9
	9	55.2±21.8	54.1±15.5	62.6±13.3	53.7±17.2
	10	48.6±17.7	59.4±12.3	63.6±15.2 *	62.6±7.5

Data taken from Table 18, pp. 132-139, MRID 46153302.

[a] Values are given as Mean ± Standard Deviation, with group size [N] as indicated.

Significantly different from control: * $p<0.05$, ** $p<0.01$.

c) **Auditory startle reflex habituation:** Results of the auditory startle reflex habituation testing are given in Table 11a (startle amplitude) and Table 11b (time to maximum amplitude). High-dose males had increased mean startle amplitudes during block numbers 2 through 5 on PND 23, although habituation over successive trial blocks was still seen. Habituation was seen over successive trial blocks in all groups on both days and in general appeared to be more pronounced on PND 23 than on PND 61. The mean times to maximum amplitude of the treated groups were similar to those of controls except for one statistically significant increase in mid-dose females during block number 4 on PND 23.

Table 11a. Auditory startle amplitude (V) [a]					
Test Day/Block		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
Males					
PND 23 [N=11, 10, 10, 5]	1	362.5±151.3	463.9±215.6	451.6±108.0	459.0±124.4
	2	237.0±57.1	291.9±67.5	258.7±55.1	331.9±90.1 *
	3	221.1±64.4	272.7±50.9	271.0±54.3	326.7±127.5 **
	4	199.5±65.8	242.7±37.3	224.1±44.6	284.6±73.2 **
	5	179.8±72.6	227.1±44.2	218.7±63.7	249.2±44.6 *
PND 61 [N=11, 10, 10, 5]	1	1451.9±168.0	1397.4±517.0	1203.7±291.6	1194.8±214.1
	2	976.5±263.9	973.4±245.9	1115.5±360.9	1184.5±169.3
	3	897.9±216.5	962.6±225.8	996.0±233.4	938.7±235.3
	4	899.1±254.5	940.0±444.6	984.9±250.9	1050.2±233.6
	5	885.5±273.2	959.3±579.4	753.1±344.2	916.8±210.7
Females					
PND 23 [N=11, 11, 10, 7]	1	383.0±152.8	392.7±115.3	382.8±107.9	320.8±70.8
	2	286.8±80.4	282.3±56.3	283.6±64.4	325.6±198.5
	3	239.8±44.4	238.1±63.2	280.4±92.9	240.8±101.4
	4	246.3±46.9	228.3±57.6	215.1±78.2	245.1±112.9
	5	227.3±39.7	210.1±70.7	214.8±56.0	222.2±109.0
PND 61 [N=11, 11, 10, 7]	1	979.8±380.3	1053.4±189.2	1206.5±290.1	1103.2±310.7
	2	921.4±266.8	1034.1±247.1	1129.8±287.8	995.0±436.2
	3	926.0±290.9	945.9±330.4	994.4±317.0	788.6±231.1
	4	698.0±241.4	758.7±278.2	771.1±362.8	763.7±98.0
	5	837.9±327.1	694.7±164.7	730.5±247.3	790.5±180.3

Data taken from Table 19, pp. 140-143, MRID 46153302.

[a] Values given as Mean ± Standard Deviation, with group size (N) as indicated.

Significantly different from control: * $p<0.05$; $p<0.01$.

Table 11b. Auditory startle reflex: time to maximum startle amplitude (msec) [a]					
Test Day/Block		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
Males					
PND 23 [N=11, 10, 10, 5]	1	25.8±4.9	28.6±11.6	29.6±5.1	22.7±3.1
	2	21.6±2.9	21.2±3.7	22.6±3.6	19.5±1.1
	3	21.3±3.1	21.7±4.8	20.7±1.7	20.4±2.8
	4	21.7±3.3	20.7±3.1	20.1±2.5	19.1±1.2
	5	21.0±2.5	20.1±2.7	20.7±3.2	20.6±1.4
PND 61 [N=11, 10, 10, 5]	1	25.3±5.8	24.2±6.3	22.8±2.0	23.9±3.5
	2	22.3±3.5	22.9±3.7	21.4±2.0	21.5±2.7
	3	22.7±3.0	23.2±3.9	22.1±2.8	23.5±3.7
	4	22.7±2.8	24.3±2.9	22.3±2.9	22.5±4.5
	5	22.9±2.9	25.3±3.7	23.9±5.1	22.7±3.1
Females					
PND 23 [N=11, 11, 10, 7]	1	29.0±7.6	26.4±6.1	25.4±5.1	24.5±4.8
	2	21.1±3.3	21.6±3.6	21.9±5.6	24.3±5.9
	3	20.0±2.2	22.7±5.8	23.3±5.4	22.9±3.8
	4	19.2±1.1	20.5±2.6	21.8±3.3 *	20.5±1.9
	5	19.8±2.6	19.8±1.6	21.6±3.8	20.0±1.4
PND 61 [N=11, 11, 10, 7]	1	24.8±4.4	25.0±3.9	24.3±2.6	23.8±3.4
	2	23.1±8.8	25.0±7.3	21.1±2.6	22.1±4.3
	3	21.4±4.4	22.7±1.8	21.5±2.4	22.8±2.8
	4	23.3±5.4	23.7±3.1	22.8±3.3	21.3±3.9
	5	25.9±7.1	22.1±3.2	23.1±2.1	23.2±2.6

Data taken from Table 20, pp. 144-147, MRID 46153302.

[a] Values given as Mean ± Standard Deviation, with group size [N] as indicated.

Significantly different from control: * $p<0.05$; $p<0.01$

d) Learning and memory testing: Selected data from the water maze testing are given in Tables 12a-b and 13a-b. There were no treatment-related effects on swimming ability (speed), as evaluated by comparison of mean straight channel times. Learning was evident in all groups at both time points as 58-77% decrease in swim time for Trial 6 on the first day compared to the groups' respective swim times for Trial 1 on the first day. Memory was evident in all groups at both time points as a ≥33% decrease in the Trial 1 swim time on the

second day of testing compared to the Trial 1 swim time on the first day of testing. However, the significantly increased Trial 1 swim time in high-dose males on PND 62 (compared to controls on the same day) is indicative of some degree of memory impairment in that group.

Compared to controls, on PNDs 27 and 62 high-dose males had significantly decreased percentages of successful trials at the lowest individualized cut-off time (i.e. at a cut-off time equal to the straight-channel time of the individual animal multiplied by 1.0; abbreviated as "SCT×1.0"). In fact, at both time points, the high-dose males had lower percentages of successful trials at "SCT×1.0" on the second day of testing than they did on the first. These differences may indicate that the high-dose males are taking longer to complete their trials for a reason other than decreased swim time. No effects were observed in females.

Table 12a. Selected water maze performance parameters for male offspring at postnatal days 24 and 27. [a]

Session/Parameter		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
PND 24	Swim time (seconds):				
	Trial 1	15.63±7.54	14.75±7.46	15.67±7.80	14.88±7.29
	Trial 6	4.98±2.24	4.77±3.12	5.96±2.79	6.19±4.04
	Straight Channel	4.87±3.96	3.38±0.98	3.84±1.82	4.28±2.34
	% Successful Trials: [b]				
	Cut-off time = 3 sec	3.6±8.6	9.8±18.7	4.6±12.5	7.7±11.0
	Cut-off time = SCT×1.0 [c]	24.6±30.1	13.7±15.9	12.0±22.0	23.1±32.3
	Cut-off time = SCT×1.5 [d]	50.0±27.1	44.1±20.4	35.2±29.1	46.2±33.4
	Cut-off time = SCT×2.0 [e]	60.1±26.0	55.9±16.6	57.4±25.7	59.0±28.6
PND 27	Swim time (seconds):				
	Trial 1	6.32±3.38	8.61±4.69	7.14±3.69	6.21±3.33
	Straight Channel	3.73±1.54	3.67±1.18	3.69±1.52	3.49±1.42
	% Successful Trials: [b]				
	Cut-off time = 3 sec	21.7±22.7	12.7±18.2	31.5±29.1	19.2±28.7
	Cut-off time = 4 sec	57.2±24.0	52.0±22.7	47.2±28.2	44.9±33.6
	Cut-off time = 5 sec	71.0±19.6	62.7±22.5	63.0±26.5	53.8±29.8
	Cut-off time = 6 sec	78.3±18.4	70.6±19.1	74.1±18.3	65.4±24.0
	Cut-off time = 7 sec	84.1±17.3	77.5±16.6	85.2±12.6	74.4±18.8
	Cut-off time = 8 sec	88.4±12.7	85.3±13.0	88.0±11.2	78.2±17.2 *
	Cut-off time = 9 sec	91.3±12.2	88.2±9.8	92.6±10.3	82.1±14.4 *
	Cut-off time = 10 sec	92.8±11.0	91.2±8.6	96.3±7.1	88.5±10.5
	Cut-off time = SCT×1.0 [c]	37.0±29.7	34.3±29.7	34.3±35.9	14.1±22.4 *
	Cut-off time = SCT×1.5 [d]	71.0±27.2	63.7±23.0	63.0±27.1	64.1±28.7
	Cut-off time = SCT×2.0 [e]	81.2±20.9	76.5±21.3	77.8±17.1	71.8±24.9

Data taken from Tables 21 and 22, pp. 148-155 and 156-171, respectively, MRID 46153302

[a] Values are given as Mean ± Standard Deviation, with N = 23, 17, 18, and 13

[b] A successful trial is one that is completed in less than the given cut-off time. The percentage of trials meeting a specific criterion was calculated for each individual animal and used to determine the group mean for that criterion.

[c, d, e] Cut-off times equal to 1.0, 1.5, and 2.0 times the individual animal's straight-channel time.

Significantly different from control: * $p<0.05$; ** $p<0.01$.

Table 12b. Selected water maze performance parameters for female offspring at postnatal days 24 and 27. [a]

Session/Parameter		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
PND 24	Swim time (seconds):				
	Trial 1	14.32±5.47	15.00±8.29	13.82±4.80	14.94±7.44
	Trial 6	5.06±3.67	4.16±2.05	4.72±2.11	5.61±3.95
	Straight Channel	4.00±1.94	3.97±2.71	4.26±1.54	4.88±3.64
	% Successful Trials: [b]				
	Cut-off time = 3 sec	12.7±21.7	15.1±25.2	7.9±18.0	14.1±17.8
	Cut-off time = SCT×1.0 [c]	28.6±33.0	18.3±27.8	23.8±28.7	34.6±36.9
	Cut-off time = SCT×1.5 [d]	54.8±31.2	51.6±24.7	48.4±26.8	57.7±26.9
	Cut-off time = SCT×2.0 [e]	60.3±26.6	61.1±21.9	67.5±22.7	65.4±25.9
PND 27	Swim time (seconds):				
	Trial 1	8.10±5.03	7.26±2.87	7.99±5.36	9.84±7.12
	Straight Channel	3.43±1.26	4.09±1.68	4.00±1.99	3.04±0.54
	% Successful Trials: [b]				
	Cut-off time = 3 sec	21.4±28.0	28.6±24.3	11.9±17.6	21.8±24.9
	Cut-off time = 4 sec	55.6±24.9	59.5±20.8	39.7±22.7 *	55.1±23.0
	Cut-off time = 5 sec	65.9±20.1	66.7±17.5	60.3±23.8	66.7±19.2
	Cut-off time = 6 sec	72.2±16.9	75.4±16.3	70.6±15.7	74.4±17.5
	Cut-off time = 7 sec	78.6±16.8	83.3±15.8	77.8±16.1	79.5±12.1
	Cut-off time = 8 sec	84.9±13.8	84.9±14.8	82.5±15.3	85.9±11.5
	Cut-off time = 9 sec	86.5±12.5	87.3±12.8	84.9±13.8	87.2±10.0
	Cut-off time = 10 sec	88.1±9.3	91.3±11.3	89.7±11.2	88.5±10.5
	Cut-off time = SCT×1.0 [c]	30.2±33.2	44.4±30.4	30.2±34.8	35.4±20.9
	Cut-off time = SCT×1.5 [d]	63.5±23.3	71.4±21.2	61.1±25.5	62.8±19.4
	Cut-off time = SCT×2.0 [e]	75.4±20.2	82.5±20.1	75.4±19.5	74.4±16.1

Data taken from Tables 21 and 22, pp. 148-155 and 156-171, respectively, MRID 46153302.

[a] Values are given as Mean ± Standard Deviation, with N = 21, 21, 21, and 13

[b] A successful trial is one that is completed in less than the given cut-off time. The percentage of trials meeting a specific criterion was calculated for each individual animal and used to determine the group mean for that criterion.

[c, d, e] Cut-off times equal to 1.0, 1.5, and 2.0 times the individual animal's straight-channel time.

Significantly different from control: * $p<0.05$, ** $p<0.01$.

Table 13a. Selected water maze performance parameters for male offspring at postnatal days 59 and 62. [a]

Session/Parameter		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
PND 59	Swim time (seconds):				
	Trial 1	13.75±5.83	16.90±5.26	14.50±6.00	13.19±5.89
	Trial 6	5.52±4.02	3.81±1.34	4.07±1.67	5.24±3.21
	Straight Channel	3.40±0.78	3.40±0.73	3.05±0.61	3.67±0.81
	% Successful Trials: [b]				
	Cut-off time = 3 sec	8.7±14.5	12.5±22.9	9.2±16.6	1.5±5.0
	Cut-off time = SCT×1.0 [c]	18.3±26.8	20.0±20.7	11.7±18.0	16.7±21.1
	Cut-off time = SCT×1.5 [d]	50.8±24.4	55.8±15.6	45.8±24.1	54.5±22.5
	Cut-off time = SCT×2.0 [e]	63.5±20.2	67.5±14.8	60.8±21.8	63.6±24.5
PND 62	Swim time (seconds):				
	Trial 1	4.51±2.04	4.65±2.09	4.92±2.61	7.09±2.95 **
	Straight Channel	3.07±0.49	3.30±0.88	2.88±0.76	2.85±0.39
	% Successful Trials: [b]				
	Cut-off time = 3 sec	26.2±25.0	18.3±20.2	26.7±25.6	15.2±20.4
	Cut-off time = 4 sec	50.8±22.0	52.5±23.7	50.8±23.2	37.9±24.8
	Cut-off time = 5 sec	64.3±19.2	60.0±23.2	64.2±20.4	57.6±25.1
	Cut-off time = 6 sec	73.8±14.5	70.0±19.9	71.7±17.2	62.1±22.5
	Cut-off time = 7 sec	80.2±11.3	75.8±19.8	78.3±15.4	74.2±17.3
	Cut-off time = 8 sec	85.7±10.9	79.2±18.6	83.3±16.2	77.3±20.1
	Cut-off time = 9 sec	88.1±9.3	80±19.2	91.7±12.7	80.3±16.4
	Cut-off time = 10 sec	91.3±10.0	82.5±15.7 *	92.5±10.1	84.8±15.7
	Cut-off time = SCT×1.0 [c]	26.2±26.7	25.8±27.8	14.2±21.8	9.1±20.2 *
	Cut-off time = SCT×1.5 [d]	61.1±16.1	55.8±23.7	56.7±24.4	43.9±26.1
	Cut-off time = SCT×2.0 [e]	73.8±15.4	70.0±22.0	71.7±19.6	60.6±20.1

Data taken from Tables 21 and 22, pp. 148-155 and 156-171, respectively, MRID 46153302

[a] Values are given as Mean ± Standard Deviation, with N = 21, 20, 20, and 11.

[b] A successful trial is one that is completed in less than the given cut-off time. The percentage of trials meeting a specific criterion was calculated for each individual animal and used to determine the group mean for that criterion.

[c, d, e] Cut-off times equal to 1.0, 1.5, and 2.0 times the individual animal's straight-channel time.

Significantly different from control: * $p<0.05$; ** $p<0.01$

Table 13b. Selected water maze performance parameters for female offspring at postnatal days 59 and 62. [a]

Session/Parameter		Dose (mg/kg bw/day)			
		Control	0.1	1.0	7.5
PND 59	Swim time (seconds):				
	Trial 1	15.42±5.31	13.56±5.73	14.35±5.55	16.38±8.46
	Trial 6	4.08±2.42	4.49±2.17	6.02±4.65	6.45±5.23
	Straight Channel	3.01±0.72	4.03±2.95	3.71±2.11	3.28±0.84
	% Successful Trials: [b]				
	Cut-off time = 3 sec	15.1±27.3	22.5±20.4	8.3±16.4	3.8±7.3
	Cut-off time = SCT×1.0 [c]	11.1±13.3	23.3±28.3	13.9±23.0	9.0±11.0
	Cut-off time = SCT×1.5 [d]	46.8±23.3	58.3±20.6	38.9±28.0	43.6±23.1
	Cut-off time = SCT×2.0 [e]	56.3±19.3	75.0±15.8 **	56.5±24.3	60.3±16.0
PND 62	Swim time (seconds):				
	Trial 1	4.95±2.71	5.17±3.62	7.16±4.82	4.93±2.60
	Straight Channel	3.10±1.24	3.82±3.08	3.22±1.05	2.70±0.33
	% Successful Trials: [b]				
	Cut-off time = 3 sec	27.8±21.9	26.7±27.8	25.9±29.3	23.1±23.1
	Cut-off time = 4 sec	56.3±19.3	55.0±24.8	50.9±27.1	47.4±26.2
	Cut-off time = 5 sec	67.5±22.0	65.8±21.9	62.0±23.4	61.5±29.2
	Cut-off time = 6 sec	75.4±20.8	72.5±18.2	67.6±25.2	65.4±28.4
	Cut-off time = 7 sec	81.0±21.3	76.7±15.7	73.1±20.7	67.9±29.2
	Cut-off time = 8 sec	84.9±16.6	78.3±17.2	82.4±19.4	74.4±26.0
	Cut-off time = 9 sec	85.7±16.1	82.5±15.7	88.0±13.8	80.8±24.4
	Cut-off time = 10 sec	88.1±15.9	87.5±17.0	88.0±13.8	83.3±21.5
	Cut-off time = SCT×1.0 [c]	20.6±24.1	25.8±30.8	25.0±29.8	6.4±10.8
	Cut-off time = SCT×1.5 [d]	63.5±20.8	60.8±25.5	55.6±28.0	52.6±27.1
	Cut-off time = SCT×2.0 [e]	73.0±21.4	73.3±20.5	73.1±22.2	62.8±29.8

Data taken from Tables 21 and 22, pp. 148-155 and 156-171, respectively, MRID 46153302.

[a] Values are given as Mean ± Standard Deviation, with N = 21, 20, 18, and 13.

[b] A successful trial is one that is completed in less than the given cut-off time. The percentage of trials meeting a specific criterion was calculated for each individual animal and used to determine the group mean for that criterion.

[c, d, e] Cut-off times equal to 1.0, 1.5, and 2.0 times the individual animal's straight-channel time.

Significantly different from control: * $p \leq 0.05$, ** $p \leq 0.01$.

5. Postmortem results:

a) Brain weight: Brain weight data are given in Table 14. There was no evidence of a treatment-related effect on whole brain or cerebellum weights at either time point.

Table 14. Brain weight data. [a]				
Study Day/Parameter	Dose (mg/kg bw/day)			
	Control	0.1	1.0	7.5
Males				
PND 12: [b] [N]	[11-12]	[10]	[10]	[6]
Terminal body weight (g)	22.7±2.8	22.6±2.1	22.7±2.9	22.6±4.5
Brain weight (g)	1.12±0.07	1.13±0.05	1.11±0.04	1.11±0.11
Brain/BW ratio (%) [c]	4.89±0.46	4.98±0.36	4.96±0.76	5.06±0.77
Cerebellum weight (g)	0.111±0.012	0.105±0.007	0.107±0.009	0.106±0.014
Cerebellum/BW ratio (%) [c]	0.487±0.075	0.469±0.043	0.478±0.082	0.477±0.044
PND 63: [N]	[12]	[10]	[11]	[11]
Terminal body weight (g)	330.3±16.5	341.5±22.9	351.7±26.7	357.3±19.2
Brain weight (g)	1.97±0.07	1.96±0.07	1.97±0.07	1.97±0.04
Brain/BW ratio (%) [c]	0.60±0.03	0.57±0.03	0.56±0.05	0.55±0.03
Cerebellum weight (g)	0.289±0.016	0.294±0.021	0.295±0.012	0.290±0.025
Cerebellum/BW ratio (%) [c]	0.087±0.006	0.086±0.003	0.084±0.007	0.082±0.009
Females				
PND 12: [b] [N]	[11]	[10]	[11]	[7]
Terminal body weight (g)	21.5±1.8	20.7±2.3	22.7±2.2	23.6±2.3
Brain weight (g)	1.07±0.05	1.04±0.03	1.07±0.03	1.08±0.06
Brain/BW ratio (%) [c]	5.01±0.37	5.11±0.62	4.76±0.46	4.62±0.28
Cerebellum weight (g)	0.103±0.011	0.095±0.014	0.102±0.011	0.100±0.011
Cerebellum/BW ratio (%) [c]	0.480±0.053	0.470±0.108	0.450±0.039	0.426±0.042
PND 63: [N]	[11]	[11]	[10]	[10]
Terminal body weight (g)	214.3±21.4	223.9±12.9	230.2±12.8	217.4±18.1
Brain weight (g)	1.8±0.06	1.83±0.06	1.85±0.08	1.83±0.09
Brain/BW ratio (%) [c]	0.85±0.08	0.82±0.06	0.80±0.02	0.84±0.07
Cerebellum weight (g)	0.267±0.017	0.268±0.022	0.272±0.021	0.277±0.008
Cerebellum/BW ratio (%) [c]	0.126±0.015	0.120±0.013	0.118±0.008	0.128±0.011

Data taken from Table 23, pp. 172-177, MRID 46153302

[a] Values given as Mean ± Standard Deviation (where provided), with group sizes [N] as indicated.

[b] Whole brain and cerebellum weights were measured after fixation in pups of this age.

[c] Mean Brain/BW and Cerebellum/BW ratios were not subjected to statistical analysis.

b) **Macroscopic examination:** There were no abnormal gross findings in any of the F_1 animals killed at the interim sacrifice on PND 12. Among the F_1 animals killed on PND 63 for brain weight or neuropathology/morphometry evaluation, abnormal gross findings were limited to renal pelvic dilatation in two low-dose males, and a kinked tail in one high-dose female.

Some of the F1 animals that died or were sacrificed moribund were subjected to gross necropsy. Of these, one high-dose male had a pale liver. The other findings mainly involved the respiratory tract and thoracic cavity and were consistent with mis-dosing, such as oral/nasal discharge, mottled or discolored lungs, and pulmonary and pleural adhesions. No tissues were retained and/or examined. There were no macroscopic findings involving the nervous system.

c) **Neurohistopathology:** No treatment-related effects were seen at PND 12. At PND 63, high-dose females had slightly increased incidences of minimal demyelination of the proximal (7/11 vs 4/12 for controls) and distal (4/11 vs 2/12 for controls) tibial nerves. The incidences of the peripheral nerve findings were within the provided historical control ranges. Dose-blinded re-reading of the slides was not conducted, and peripheral nerve tissues from the lower dose groups were not examined.

Mean counts of Purkinje cell bodies (per mm) in lobule 8 of the cerebellum were not affected by treatment.

d) **Morphometric evaluation:** Morphometric measurements taken in the cerebrum and brain stem are given in Tables 15a-b, and those taken in the cerebellum are given in Table 16. At PND 12, high-dose females had an increase in the thickness of the molecular layer of the prepyramidal fissure of the cerebellum, and at PND 63, high-dose males had a decrease in the thickness of the inner granular layer of the prepyramidal fissure of the cerebellum. These findings are considered possibly treatment-related and adverse. There were no alterations in the cortical cell layers of the preculminate fissure or in the length and height of the cerebellum.

Changes in the morphometry of the cerebrum/brain stem slices were seen in high-dose animals of both sexes at PND 63. High-dose males and females had an increased hippocampal width at Level 4 and increased widths of the dentate gyrus of the hippocampus on Levels 4 and 5. Both sexes also had an increase in one of the piriform cortex measurements: in males this was seen on Level 5, and in females this was seen on Level 4. Males had a decreased thalamus height on Level 4, and females had a decreased corpus callosum thickness on level 4.

Table 15a. Brain morphometry of cerebrum and brainstem in male offspring (mm). [a]					
Parameter Description and [Number]		Dose Level (mg/kg bw/day)			
		Control	7.5	Control	7.5
		PND 12 [N = 12 and 6]		PND 63 [N = 11 and 9-10]	
Frontal Cortex					
Height - Level 2	[2A]	5.66±0.32	5.64±0.38	6.60±0.61	6.65±0.23
Width - Level 2	[2B]	4.53±0.30	4.52±0.27	5.13±0.77	4.82±0.28
Dorsal Cortex:					
Thickness - Level 3	[3A]	1.46±0.07	1.46±0.13	1.34±0.08	1.29±0.14
Thickness - Level 3	[3B]	1.60±0.08	1.57±0.08	1.67±0.13	1.65±0.17
Thickness - Level 4	[4A]	1.39±0.09	1.40±0.14	1.49±0.09	1.41±0.09
Thickness - Level 5	[5A]	1.22±0.09	1.22±0.12	1.39±0.14	1.41±0.07
Piriform Cortex					
Thickness - Level 3	[3C]	1.23±0.10	1.18±0.09	1.05±0.11	1.14±0.15
Thickness - Level 4	[4B]	1.14±0.10	1.09±0.14	1.05±0.12	1.15±0.10
Thickness - Level 5	[5B]	1.14±0.09	1.18±0.07	1.06±0.11	1.17±0.08 *
Hippocampus:					
Length - Level 3	[3D]	3.12±0.29	3.21±0.33	2.36±0.18	2.39±0.30
Length - Level 4	[4G]	4.07±0.24	4.14±0.28	3.61±0.38	3.92±0.27
Width - Level 5	[5E]	1.47±0.12	1.48±0.06	1.44±0.08	1.57±0.09 *
Dentate gyrus length - Level 4	[4J]	1.53±0.13	1.59±0.30	1.67±0.15	1.61±0.19
Dentate gyrus width - Level 4	[4H]	0.57±.05	0.61±0.04	0.54±0.05	0.65±0.04 *
Dentate gyrus width - Level 5	[5D]	0.80±0.08	0.80±0.05	0.65±0.04	0.78±0.05 **
Corpus Callosum:					
Thickness - Level 4	[4C]	0.57±0.09	0.56±0.07	0.36±0.06	0.32±0.05
Thalamus:					
Height - Level 4	[4D]	5.49±0.26	5.52±0.17	5.39±0.27	4.96±0.46 *
Width - Level 4	[4E]	8.16±0.47	8.41±0.26	8.65±0.39	8.75±0.37
Width - Level 5	[5C]	7.39±0.45	7.74±0.27	7.98±0.25	8.02±0.31
Thalamus/Cortex:					
Overall width - Level 4	[4F]	13.98±0.67	14.05±0.61	14.89±.38	14.84±0.36

Data taken from Table 27, pp. 183-206, MRID 46153302

[a] Values given as Mean ± Standard Deviation, with group sizes [N] as indicated.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

Table 15b. Brain morphometry of cerebrum and brainstem in female offspring (mm). [a]					
Parameter Description and [Number]		Dose Level (mg/kg bw/day)			
		Control	7.5	Control	7.5
		PND 12 [N = 11 and 7]		PND 63 [N = 12 and 11]	
Frontal Cortex:					
Height - Level 2	[2A]	5.58±0.48	5.56±0.19	6.57±0.25	6.52±0.26
Width - Level 2	[2B]	4.47±0.40	4.28±0.21	4.83±0.31	4.70±0.41
Dorsal Cortex:					
Thickness - Level 3	[3A]	1.42±0.08	1.42±0.10	1.32±0.11	1.29±0.13
Thickness - Level 3	[3B]	1.58±0.08	1.58±0.09	1.68±0.10	1.68±0.10
Thickness - Level 4	[4A]	1.37±0.08	1.38±0.12	1.38±0.10	1.39±0.10
Thickness - Level 5	[5A]	1.20±0.06	1.24±0.08	1.33±0.08	1.34±0.09
Piriform Cortex:					
Thickness - Level 3	[3C]	1.12±0.08	1.19±0.08	1.09±0.12	1.15±0.08
Thickness - Level 4	[4B]	1.15±0.08	1.16±0.09	1.08±0.13	1.21±0.11 *
Thickness - Level 5	[5B]	1.11±0.08	1.10±0.09	1.09±0.10	1.16±0.08
Hippocampus:					
Length - Level 3	[3D]	3.04±0.25	2.99±0.48	2.59±0.17	2.54±0.27
Length - Level 4	[4G]	4.13±0.21	4.16±0.13	3.70±0.30	3.88±0.35
Width - Level 5	[5E]	1.48±0.07	1.56±0.11	1.43±0.10	1.53±0.06 **
Dentate gyrus length - Level 4	[4J]	1.59±0.12	1.52±0.13	1.61±0.13	1.71±0.20
Dentate gyrus width - Level 4	[4H]	0.58±0.05	0.57±0.06	0.58±0.08	0.68±0.05 *
Dentate gyrus width - Level 5	[5D]	0.77±0.06	0.81±0.06	0.66±0.06	0.76±0.04 **
Corpus Callosum:					
Thickness - Level 4	[4C]	0.56±0.13	0.56±0.15	0.37±0.07	0.31±0.04 *
Thalamus:					
Height - Level 4	[4D]	5.66±0.28	5.56±0.26	5.32±0.29	5.43±0.27
Width - Level 4	[4E]	8.24±0.34	8.17±0.48	8.58±0.36	8.60±0.27
Width - Level 5	[5C]	7.58±0.33	7.49±0.51	7.71±0.29	7.79±0.09
Thalamus/Cortex					
Overall width - Level 4	[4F]	13.91±0.33	13.91±0.60	14.44±0.64	14.75±0.45

Data taken from Table 27, pp. 183-206, MRID 46153302.

[a] Values given as Mean ± Standard Deviation, with group sizes [N] as indicated.
Significantly different from control: * $p<0.05$ ** $p<0.01$.

Table 16. Brain morphometry of cerebellum [a]

Parameter Description and [Number]	Dose Level (mg/kg bw/day)			
	Control	7.5	Control	7.5
	Males		Females	
PND 12				
[Number Examined]	[12]	[6]	[11]	[6]
Height (mm) [8H]	3.84±0.20	3.91±0.32	3.81±0.29	3.87±0.22
Length (mm) [8L]	4.35±0.19	4.20±0.25	4.37±0.40	4.39±0.35
Thickness of cerebellar cortex layers (μm)				
Preculminate Fissure:				
Molecular layer [8PCFM]	75.6±9.0	76.5±11.5	83.5±10.5	79.4±6.4
Outer granular layer [8PCFO]	39.9±3.7	41.1±4.0	40.0±5.4	35.9±6.3
Inner granular layer [8PCFI]	148±20	136±23	157±9	155±25
Prepyramidal Fissure:				
Molecular layer [8PPFM]	62.0±6.2	62.2±10.2	58.2±8.2	70.1±10.2 *
Outer granular layer [8PPFO]	44.2±6.8	48.8±4.5	49.1±10.1	48.7±4.2
Inner granular layer [8PPFI]	145±29	132±24	134±11	146±21
PND 63				
[Number Examined]	[11]	[10]	[11-12]	[11]
Height (mm) [8H]	5.45±0.21	5.55±0.21	5.31±0.30	5.33±0.28
Length (mm) [8L]	6.90±0.41	7.10±0.28	6.80±0.38	6.82±0.42
Thickness of cerebellar cortex layers (μm)				
Preculminate Fissure:				
Molecular layer [8PCFM]	214.5±24.9	216.8±20.1	212.5±12.4	212.1±20.2
Inner granular layer [8PCFI]	188±11	178±25	179±30	165±26
Prepyramidal Fissure:				
Molecular layer [8PPFM]	207.5±14.9	210.0±17.5	198.0±15.8	203.0±14.7
Inner granular layer [8PPFI]	157±26	134±20 *	153±19	139±24

Data taken from Table 27 pp. 183-206, MRID 46153302.

[a] Values given as Mean ± Standard Deviation, with group sizes [N] as indicated.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that there were no treatment-related effects on the F_0 parent females. The study author also concluded that no evidence of toxicity, including neurotoxicity, was seen in the F_1 offspring. Increased values for several morphometric measurements in the hippocampus were considered treatment-related but not adverse.

B. REVIEWER COMMENTS:

Two total litter resorptions in the high-dose group may be treatment-related. Inordinately high non-treatment-related pup mortality (and total litter losses) during LD 1-5 make it difficult to distinguish any treatment-related pup mortality that may have occurred during that time. Slightly higher than expected offspring mortality was also observed during LD 5-22.

Offspring toxicity manifested as neurobehavioral changes in high-dose males. This group had increased mean startle amplitudes on PND 23 (for Blocks 2-5). Spatial memory impairment was evident at retention testing on PND 62 as an increased Trial 1 swim time compared to controls, although the results did indicate that at least some memory was present. At retention testing on PNDs 27 and 62, this group had decreased percentages of successful trials both compared to controls and compared to their own previous results on the first day of testing.

Brain morphometry changes in high-dose animals at PND 63 provided additional evidence of possible toxicity. Both sexes had increased hippocampus width at Level 5, increased width of the dentate gyrus of the hippocampus on Level 4 and Level 5, and increased piriform cortex thickness, seen on Level 5 in males and on Level 4 in females. Males had a decreased thickness of the inner granular layer of the prepyramidal fissure, and decreased thalamus height on Level 4. Females had decreased corpus callosum thickness on Level 4. The morphometric changes in the hippocampus (including dentate gyrus), thalamus, and cerebellum may be related to the memory impairment in males and also correlate to the increased startle response in males (on PND 23), although the time course was different. No neurobehavioral correlates were detected for the decreased corpus callosum thickness in females.

An increased thickness of the molecular layer of the prepyramidal fissure in high-dose females on PND 12 was considered possibly treatment-related although of unknown significance.

The incidence and/or severity of demyelination of several peripheral nerves in high-dose females at PND 63 were slightly increased but remained within historical control ranges. These changes are common findings and were considered to be incidental to treatment even though dose-blinded re-reading of the slides was not conducted, and peripheral nerve tissues from the lower dose groups were not examined.

Discrepancies between the conclusions of the reviewer and those of the study author concerned the increased startle amplitude in high-dose males on PND 23, the results of the water maze testing, and whether or not the effects on brain morphometry were treatment-related and/or adverse.

According to the study author, increases in mean startle amplitude in high-dose males on PND 23 during blocks 2-5 were due to high values in two animals that were tested that day and were also affected by greater body weight in the high-dose animals than in controls. Although the reviewer agrees that body weight can affect startle amplitude, it is unlikely that the cited 11.8% difference in body weight would result in 39-48% increases in mean startle response, and it is even less likely that it would do so during Blocks 2-5 without having a similar effect during Block 1.

The reviewer interpreted the results of the water maze testing in a different manner than did the study author. The reviewer disagrees with the study author's assumption that changes seen in only one sex and/or at only one time point cannot be treatment-related. Moreover, it is the opinion of the reviewer that a treatment-related difference can be evident using one method of analysis but not be evident using the other method of analysis.

The reviewer disagrees with the study author's implication that a morphometric change seen in only one sex or at only one level cannot be treatment-related. The reviewer also disagrees with the study author's statement that treatment-related morphometric changes in the hippocampus were not adverse effects because they were increases rather than decreases.

The inordinate pup mortality in all groups including controls during lactation was most pronounced during PND 1-5 and is indicative of compromised health status or some other problem with the animals on study. The high numbers of total litter losses resulted in too few high-dose F_1 litters to allocate the minimum number of offspring to all endpoints. It is the opinion of the reviewer that the data from the motor activity testing are inadequate to preclude a treatment-related effect on motor activity. The absence of habituation during motor activity testing indicates a problem with the testing procedure and/or a continued problem with animal health. Likewise, the results of the FOB are inadequate to assess the evaluated parameters because the same animals were not evaluated at all time points. However, adequate numbers of control animals were evaluated for each measured parameter. For this reason, the study is tentatively classified as **Unacceptable (not-upgradable).** Further discussion of the study deficiencies is included below.

C. STUDY DEFICIENCIES:

Major deficiencies include the following:

- The high pup mortality in controls and high dose groups during lactation period, LD1-5 is of concern since this finding is indicative of compromised health status of the animals or some other technical difficulties with the conduct of the study.

- The high numbers of total litter losses in the high dose group contributed to too few F_1 litters to allocate the minimum number of offspring to neurobehavioral endpoints. In the high-dose group, only 7/sex were assigned for motor activity evaluation, only 5-7/sex were assigned for auditory startle habituation, and only 6-7/sex were assigned for PND 62 brain weight. Only seven low-dose females were evaluated for motor activity even though this group had 21 acceptable litters available for experiments.

- The absence of habituation during motor activity testing indicates a problem with the testing procedure and/or a continued problem with animal health.

- The offspring functional observational battery assessments did not consistently evaluate the same individual animals at all scheduled time points. Some instances appeared to be a later assignment of an additional animal as a substitute for one that had died; this is understandable, but it should have been documented in the study report. Occurrences when individuals were evaluated at only one or two time points or when individuals were evaluated at *most* time points, with missing time points occurring non-consecutively in the middle of the study are unacceptable.

- The experimental details on the auditory startle reflex and the motor activity are missing. A description (or make and model number) of the monitoring devices for the motor activity was not provided. Also, there was no description of the equipment used, environmental conditions, length (msec) and intensity (dB) of sound, or the length of the interval between trials for auditory startle reflex measurement.

- The morphometric data for the low and mid dose groups were not reported. The high dose group had morphometric changes in the thalamus, hippocampus and to some extent in cerebral cortex and cerebellum. It is not known if these effects were also observed in lower dose groups.

- Additionally, the data were presented in a disorganized manner. This made it difficult and time consuming to follow the disposition of individual animals and their litters and evaluate parameters such as the survival of F_1 offspring after PND 5.

APPENDIX: Preliminary Developmental Neurotoxicity Study - Rat; Range-finding.

TEST MATERIAL (PURITY): Dichlorvos, technical material (99.0% a.i.; batch #ST120700)

CITATION: G. Milburn (2003) Dichlorvos: preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory report number CTL/RR00885/Regulatory/Report, October 13, 2003. MRID 46153301. Unpublished.

EXECUTIVE SUMMARY:

In a preliminary developmental neurotoxicity study (MRID 46153301) Dichlorvos (99.0% a.i., batch #ST120700) was administered by gavage in de-ionized water to 15 time-mated female Alpk:AP,SD (Wistar-derived) rats per dose at dose levels of 0, 0.1, 1.0, or 7.5 mg/kg bw/day from gestation day (GD) 7 through postnatal day (PND) 22. In-life observations included maternal clinical signs, body weight, and food consumption (during gestation) and the number, survival, clinical signs, and body weight of the pups. Erythrocyte (RBC) and whole brain acetylcholinesterase (AChE) activities were measured as follows: in 5 dams/group on GD 22; in 5 dams/group on PND 22; in selected fetuses from the dams killed on GD 22 (blood from sufficient fetuses to attain adequate pooled sample volume and whole brain from 4 fetuses/sex/litter); and in 5 pups/sex/group (1 per litter where possible) on each of PNDs 2, 8, 15, and 22. Plasma AChE activity was not measured.

There were no maternal deaths during the study. Three dams had abnormal clinical signs: one control dam with piloerection on day 26; one mid-dose dam with observations of paleness (days 24-26), hunched, subdued behavior (day 26), and a total litter loss by day 26 (LD 3); and one high-dose dam with irregular breathing on days 25-27. There were no treatment-related effects on maternal food consumption, maternal body weight, or gestation length. The study author mentioned body weight decreases in high-dose dams beginning on LD 11, but these were of insufficient magnitude to be considered biologically significant (just 3-4% less than controls). **Under the conditions of this study, the LOAEL for maternal systemic toxicity (other than acetylcholinesterase inhibition) is not identified, and the NOAEL is greater than or equal to 7.5 mg/kg bw/day.**

There were no treatment-related effects on the overall proportion of pups born alive, the mean percentage of live pups per litter, or live litter size on LD 1. Pup survival, body weight, and clinical signs were unaffected by treatment. Two dams had total litter losses: one mid-dose dam had a total litter loss by LD 3, and one low-dose dam had a total litter loss (of 1 pup) by LD 2. An increased proportion of male pups in the mid-dose group (64.8% vs. 46.2% for controls; $p<0.01$) was considered incidental to treatment because there was no similar finding at the highest dose level. **Under the conditions of this study, the LOAEL for offspring toxicity (other than acetylcholinesterase inhibition) is not identified, and the NOAEL is greater than or equal to 7.5 mg/kg bw/day.**

In maternal animals, RBC AChE activity was biologically significantly inhibited at the mid- and high-dose treatment levels on GD 22 by 25% and 48%, respectively ($p<0.01$) and on LD 22 by 24% and 50%, respectively ($p<0.05$ and $p<0.01$). RBC AChE activity was also inhibited in high-

dose male and female (GD 22) fetuses by 28% ($p<0.5$) and 21% (n.s.), respectively. There were no treatment-related effects on RBC AChE activity in male or female pups. **The LOAEL for dichlorvos erythrocyte acetylcholinesterase inhibition in maternal rats is 1.0 mg/kg bw/day, with a NOAEL of 0.1 mg/kg bw/day. The LOAEL for erythrocyte acetylcholinesterase inhibition in offspring or fetuses is 7.5 mg/kg bw/day (based on male and female fetuses on GD 22), and the NOAEL is 1.0 mg/kg bw/day.**

In maternal animals, whole brain AChE activity was biologically significantly inhibited in high-dose animals on GD 22 and LD 22 by 59% and 67%, respectively ($p<0.01$). Brain AChE activity was also inhibited in high-dose male and female (GD 22) fetuses by 16% ($p<0.5$) and 21%, respectively ($p<0.01$). There were no treatment-related effects on brain AChE activity in male or female pups. **The LOAEL for brain acetylcholinesterase inhibition in maternal animals is 7.5 mg/kg bw/day, with a NOAEL of 1.0 mg/kg bw/day. The LOAEL for brain acetylcholinesterase inhibition in offspring or fetuses is 7.5 mg/kg bw/day (based on male and female fetuses on GD 22), and the NOAEL is 1.0 mg/kg bw/day.**

Based on the results of this study, dose levels of 0, 0.1, 1.0, and 7.5 mg/kg bw/day were chosen for the main study

Table 1
Parent Female and Fetal/Pup Cholinesterase Inhibition

Time Point	Compart-ment	Sex	0.1 mg/kg/day	1.0 mg/kg/day	7.5 mg/kg/day
Day 22 gestation	Brain	Parent Female	ns	ns	59%**
Day 22 gestation	Erythrocyte	Parent Female	ns	25%**	48%**
Day 22 lactation	Brain	Parent Female	ns	ns	67%**
Day 22 lactation	Erythrocyte	Parent Female	ns	24%*	50%**
Fetus	Brain	Male Female	ns ns	ns ns	16%* 21%**
Fetus	Erythrocyte	Male Female	ns ns	ns ns	28%* ns (21%)
Day 2 post partum	Brain	Male Female	ns ns	ns ns	ns ns
Day 2 post partum	Erythrocyte	Male Female	ns ns	ns ns	ns ns
Day 8 post partum	Brain	Male Female	ns ns	ns ns	ns ns
Day 8 post partum	Erythrocyte	Male Female	ns ns	ns ns	ns ns
Day 15 post partum	Brain	Male Female	ns ns	ns ns	ns ns
Day 15 post partum	Erythrocyte	Male Female	ns ns	ns ns	ns ns
Day 22 post partum	Brain	Male Female	ns ns	ns ns	ns ns
Day 22 post partum	Erythrocyte	Male Female	ns ns	ns ns	ns ns

ns = not significantly different from Control

* = Statistically significant difference from the Control group at $p<0.05$ level (Student's t-test, two sided)
** = Statistically significant difference from the Control group at $p<0.01$ level (Student's t-test, two sided)

DATA EVALUATION RECORD

DICHLORVOS/084001

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT [OPPTS 870.6300 (§83-6); OECD 426]

MRID 46239801

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
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Task Order No. 57-2004

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Toxicology Branch, Health Effects Division (7509C) Date 11/3/05
Template version 11/01

TXR#:0053832

DATA EVALUATION RECORD

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

PC CODE: 084001 **DP BARCODE:** D305082
SUBMISSION NO.: none provided

TEST MATERIAL (PURITY): Dichlorvos Technical Material (99.0% a.i.)

SYNONYMS: DDVP

CITATION: G.M. Milburn (2004) Dichlorvos: supplemental developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number CTL/RR0988/Regulatory/Report, January 28, 2004. MRID 46239801. Unpublished.

SPONSOR: Amvac Chemical Corporation.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2004, MRID 46239801, study RR0988) Dichlorvos (99.0% a.i., batch #ST120700) was administered to 30 time-mated female Alpk:AP,SD (Wistar-derived) rats per group by gavage in de-ionized water at dose levels of 0 or 7.5 mg/kg bw/day from gestation day (GD) 7 through postnatal day (PND) 7. Direct dosing of the F_1 offspring was carried out during PNDs 8-22, inclusive. **This study was conducted with a single dose to provide supplemental information to the previous study (MRID No. 46153302) where high number of whole litter loss at this dose was seen.**

On PND 5, litters were culled to 8 pups (4/sex as closely as possible), and litters containing fewer than 7 pups and/or fewer than 3 pups of each sex were removed from the study. The dams were subjected to a functional observational battery (FOB) on GDs 10 and 17 and on PNDs 2 and 9. The F_1 offspring were observed for attainment of preputial separation or vaginal patency. Animals were allocated for assessment of FOB (PNDs 5, 12, 22, 36, 46, and 61), locomotor activity (PNDs 14, 18, 22, and 60), auditory startle reflex habituation (PNDs 23 and 61), learning and memory (PND 24-27 or PND 59-62), and post mortem investigations including brain weight, neuropathology, and morphometry (PNDs 12 and 63).

No treatment-related deaths, clinical signs of toxicity, or abnormal FOB findings were observed in any maternal animals during the study. Maternal body weight, pregnancy rate, and gestation length were similar between the treated and control groups.

The maternal NOAEL is 7.5 mg/kg/day, the highest dose tested. A maternal LOAEL was not established.

The results of this study were confounded again by excessive litter loss in the control group similar to that of the previous study. In the control group a total of five dams had complete litter loss during lactation and another eight litters had insufficient numbers of pups for selection of F_1 animals. Only two treated dams had complete litter loss. The reason for the pup mortality is unknown but was also seen at the same dose (7.5 mg/kg/day) in the previous study. Therefore, it appears that the pup mortality may not be related to treatment, but rather reflect a problem with the animals or with the testing facility.

In the offspring available for evaluation, no treatment-related effects were observed on body weight, body weight gain, food consumption, developmental landmarks, FOB, motor activity, auditory startle reflex, learning and memory, brain weight, brain morphology or neuropathology.

The DNT Committee determined that the two DNT studies combined (RR0886 and RR0988) had acceptable numbers of total pups examined in the controls and high dose groups (> 35 pups/sex examined in combined studies) and, therefore, the developmental results of the combined studies could be evaluated for the NOAEL/LOAEL.

Therefore, the developmental/offspring NOAEL was determined to be 1.0 mg/kg/day (based on study RR0886) and the developmental/offspring LOAEL was 7.5 mg/kg/day (based on both studies RR0886 and RR0988) with the effect being increases in auditory startle reflex habituation Vmax in PND 23 high dose males in both studies.

This study when combined with the accompanying study is classified **Acceptable/non-guideline** and may be used for regulatory purposes. It does satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] pending review of the positive control data.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Dichlorvos

Description:	technical material: clear, colorless liquid
Batch #:	ST120700
Purity:	99.0 % a.i.
Compound Stability:	stability not reported; expiration date of October, 2003
CAS # of TGAI:	not reported
Structure:	not available

2. Vehicle and/or positive control: The vehicle was de-ionized water. No positive control was used in the current study.

3. Test animals (P):

Species:	Rat	
Strain:	Alpk:APfSD. (Wistar-derived)	
Age at study initiation:	10-12 wks	
Wt. at study initiation:	227-288 g	
Source:	Rodent Breeding Unit (RBU), Alderley Park, Macclesfield, Cheshire, UK	
Housing:	P: Individually in solid plastic cages with sawdust bedding; loose paper balls were provided as nesting materials (SI Supplies, Hazel Grove, Cheshire). F_1: in same sex groups of up to 4 animals in wire mesh cages	
Diet:	Powdered CT1 diet was available *ad libitum*.	
Water:	Water was available *ad libitum*; not otherwise described.	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	30-70%
	Air changes:	at least 15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Animals were supplied time-mated and arrived 6 days before dosing began.	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: April 1, 2003; End: December 9, 2003.

2. Study schedule: Time-mated females were randomly assigned to a control or treatment group upon arrival. The test substance was administered to the maternal animals from gestation day (GD) 7 through lactation day (LD) 7, where the day of birth was designated as postnatal day (PND) 1 or LD 1. Litter standardization and selection of F_1 pups were conducted on PND 5. The selected pups were dosed on PNDs 8 through 22 and remained on study until PND 63 (study termination). The selected pups were weaned on PND 29, at which time the maternal animals were killed and discarded.

3. **Mating procedure:** Females were naturally mated while at the supplier. The day on which spermatozoa were observed in a vaginal smear was designated as GD 1, and the females were shipped to the testing facility on this same day.

4. **Animal Assignment:** Animal assignment is given in Table 1. Twenty time-mated females were supplied on each of 3 days and assigned to the control or treated group using a randomized block design.

   Offspring were selected for use as F_1 animals at the time of litter standardization on PND 5. The offspring were allocated for use in neurobehavioral tests, brain weight determinations, and neuropathological evaluations by using one male pup and/or one female pup/litter.

TABLE 1. Study design

Experimental Parameter	Dose (mg/kg bw/day)	
	0	7.5
Maternal animals		
No. of maternal animals assigned and FOB (GDs 10 and 17; LDs 2 and 9)	30	30
Offspring		
FOB (PNDs 5, 12, 22, 36, 46, and 61)	8-11/sex	9-12/sex
Motor activity (PNDs 14, 18, 22, and 60)	8/sex	11-12/sex
Auditory startle habituation (PNDs 23 and 61)	8/sex	11-13/sex
Learning and memory (PNDs 24/27 and 59/62)	15-16/sex	22-23/sex
Brain weight: PND 12 (fixed weight) PND 63 (wet weight)	 8/sex 10/sex	 11-12/sex 12/sex
Neuropathology and Morphometry: PND 12 (immersion fixation) PND 63 (perfusion fixation)	 8/sex 11/sex	 10-12/sex 12/sex

5. **Dose selection rationale:** The single dose used in the current study was the same as the high dose in a definitive developmental neurotoxicity study (MRID 46153302). Due to a high number of whole litter losses at this dose in the definitive study, the current study was designed to provide supplemental information.

6. **Dosage administration:** All doses were administered once daily by gavage in de-ionized water at a dosing volume of 10 mL/kg bw/day, based on the individual daily body weight. Maternal animals were dosed from GD 7 through LD 7, and F_1 animals were dosed on PNDs 8 through 22.

7. **Dosage preparation and analysis:** The amount of the test material used was not adjusted to account for purity. The formulation was prepared every 4-6 days by adding sufficient de-ionized water to a weighed amount of test material. Each formulation was subdivided into aliquots for daily dosing and stored at room temperature until use. The method used to mix the formulation was not described, although the study report stated that the preparations were shaken prior to dose administration. Stability of the dosing formulation was measured in the definitive study (MRID 46153302). Triplicate samples of formulation from the first batch and from one subsequent batch (April 7 and 30, 2003) were analyzed for concentration. Homogeneity analysis was not done.

Results: Concentration Analysis: Absence of the test material was confirmed in the vehicle. Mean concentrations of the dose formulation were 106.0-112.3% of nominal.

Stability Analysis: The stability of the test article in the vehicle was noted to be satisfactory for 5 days after preparation; these data were not included.

Homogeneity Analysis: The formulation was stated as being a solution so homogeneity analysis was not done.

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:** Cage-side observations were conducted each morning and towards the end of each working day. Detailed clinical observations and body weight were recorded upon arrival, daily (immediately prior to dosing) during GD 7 through LD 7, and on LDs 15, 22, and 28 (termination).

   All maternal animals were subjected to a functional observational battery on GDs 10 and 17, and on LDs 2 and 9. The examinations were conducted in the home cage and in a standard (open) arena by an individual unaware of each animal's treatment group, and included evaluation of the parameters indicated (X) below. Additional details of the testing procedure (such as environmental conditions, duration of testing) and scoring criteria were not given. On treatment days, it was not stated whether the animals were tested before or after dosing.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Lacrimation or salivation 2) Piloerection or endophthalmus/exophthalmus, 3) Urine staining or diarrhea 4) Pupillary response to light: miosis/mydriasis 5) Degree of palpebral closure, i.e. ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements in the home cage and standard (open) arena.
X	Reactivity to general stimuli, including response to approach and touch.
X	Arousal level/alertness.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behavior, excessive or repetitive action (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as PND or LD 1. The sex, weight, and clinical condition of each pup was recorded on PNDs 1 and 5, and litters were checked daily throughout lactation for dead or abnormal pups.

On PND 5, litters were standardized to a maximum of 8 pups/litter (randomly selected 4/sex/litter, as nearly as possible), and litters with 7-8 pups and at least 3 pups of each sex remained on study as the F_1 generation. The excess pups were killed and discarded.

The F_1 litters remained with their dams until PND 29. Individual body weight and detailed clinical observations were recorded on PND 5, daily during PNDs 8-22 (immediately prior to dosing), and on PND 29.

2) **Postweaning observations:** After weaning on postnatal day 29, offspring were examined daily for mortality or clinical signs. Individual body weight and detailed clinical observations were recorded on PNDs 36, 43, 50, 57, and 63 (prior to termination).

3) **Developmental landmarks:** Beginning on PND 29, female offspring were examined daily for vaginal patency, and beginning on PND 36, male offspring were examined daily for balanopreputial separation. The age and body weight at the time of onset were recorded for each animal.

4) **Neurobehavioral evaluations:**

a) **Functional observational battery (FOB):** Selected F_1 offspring were subjected to a functional observational battery on PNDs 5, 12, 22, 36, 46, and 61. The examinations were conducted in the home cage and in a standard (open) arena by an individual who was unaware of each animal's treatment group. On treatment days the testing was done prior to dosing. The FOB for offspring assessed the same parameters as the maternal FOB with no mention of adjustment to account for developmental age. Additional details of the testing procedure (such as environmental conditions, duration of testing, and scoring criteria were not given.

In general, one male or one female was selected from each litter. However, in order to ensure that at least 10 animals per sex were examined, it was necessary to select one male and one female from some control litters.

b) **Motor activity testing:** Motor activity was evaluated in one male or one female per litter on PNDs 14, 18, 22, and 60. An automated activity recording apparatus was used to record large and small movements over the course of a 50-minute session, comprised of ten 5-minute scans. The same animals were evaluated at each time point. On treatment days (PND 14, 18, and 22), the testing was done prior to dosing. The treatment groups were counterbalanced across the cage numbers of the activity monitors and the assessments were done in a separate room in order to minimize environmental distraction. When the trials were repeated each animal was tested in the same monitoring device across test sessions. A description (or make and model number) of the monitoring devices was not provided.

c) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on one male or one female per litter on PNDs 23 and 61, using an automated system. Mean response amplitude and time to maximum amplitude on each of 5 blocks of 10 trials per session were calculated. No description of the equipment used, environmental conditions, length (msec) and intensity (dB) of sound, or the length of the interval between trials was given.

d) **Learning and memory testing:** Water maze testing was performed on PNDs 24/27 and on PNDs 59/62 to evaluate associative learning and memory. Separate groups of one animal/sex/litter were tested at each interval. Each session was comprised of 6 trials in a Y-shaped maze with one escape ladder followed by a single trial in a straight channel to evaluate swim speed. The amount of time required for the animal to find the ladder was recorded for each trial.

The criterion for a successful trial was a time less than a given cut-off value, and the following cut-off values were used: 3, 4, 5, 6, 7, 8, 9, and 10 seconds; and multiples of 1.0, 1.5, and 2.0 times the individual animal's straight-channel time. For each individual, the percentage of trials meeting a specific criterion was calculated and used to determine the group mean for that criterion.

Learning was assessed by comparing the swim times for Trials 1 and 6 on the first day of testing, and memory was assessed by comparing the swim time for Trial 1 on the second day of testing to the swim time for Trial 1 on the first day.

The inter-trial interval was not reported and there was no further description of the equipment or environmental conditions (lighting, water temperature and depth, background noise, etc.).

5) **Cholinesterase determination:** Biomarker data were not measured in the current study.

2. **Postmortem observations:**

a. **Maternal animals:** Females that failed to litter were sacrificed on nominal GD 26 by halothane vapor followed by exsanguination and subjected to a gross necropsy which included examination for pregnancy status. Dams with litters not selected as F_1 animals on PND 5 and females with total litter losses were sacrificed and discarded without examination. Maternal animals of the selected F_1 litters were sacrificed by halothane vapor followed by exsanguination on PND 29 and discarded without examination. No tissues were retained or processed for histopathological examination.

b. **Offspring:** On PND 5, the excess pups (i.e. those culled during litter standardization and litters not selected as F_1 animals) were killed and discarded without examination. Offspring that were found dead during the dosing interval (PND 8-22) were subjected to gross necropsy. Offspring that died or were killed for humane reasons prior to PND 8 or after PND 22 generally were discarded without examination. No tissues were retained from these animals.

The offspring selected for brain weight and/or neuropathological evaluation were sacrificed on PND 12 or on PND 63 and subjected to postmortem examinations as described below.

On postnatal day 12, one male or one female per litter were sacrificed by carbon dioxide exposure, and the brains from these animals were immediately exposed and immersion fixed in 10% neutral buffered formol saline. At least 24 hours after fixation whole brain and cerebellar weights were recorded, and the tissues were embedded in paraffin wax and processed in the following manner. The cerebellum was cut sagitally at midline to make 2 blocks (20 and 21) and the remainder of the brain was cut into 5 blocks by making transverse cuts at the following anatomic landmarks: the rostral edge of the olfactory bulb (level 1); the caudal edge of the olfactory bulb (level 2); the rostral edge of the median eminence (level 3); the caudal edge of the cerebral hemispheres (level 6); and the midpoint of the remaining brain stem. The blocks were sectioned, stained with hematoxylin and eosin, and examined using light microscropy.

An image analysis system (KS400) was used to make the morphometric measurements given in Table 2. The system used a light box, macro lens, and video camera, calibrated by means of a graticule, to take the measurements on levels 2-5 of the cerebrum/brainstem and to measure the height and length of the section of the cerebellum. The rest of the cerebellum measurements were made using a light microscope, calibrated by means of a stage micrometer. Measurements of width, length, and height were made over the maximum dimension of the indicated structure, and dorsal cortex measurements were made at right angles to a tangential line at the surface of the brain and extended from the meningeal surface to the inner edge of the pyramidal cells adjacent to the white matter of the external capsule. Bilateral features on the cerebrum/brainstem sections were measured on both the left and right sides unless one side was oblique or failed to show the feature in question for some other reason. The cerebellum was measured on one of the two slides, i.e. the one that provided the best sagittal section. In some cases, it was not possible to cut an adequate section for one of the levels.

The image analysis system was also used to measure the length of the Purkinje cell layer on lobule 8 of the cerebellum adjacent to the prepyramidal fissure. The number of Purkinje cell bodies in lobule 8 were counted and expressed as a function of the length of lobule 8

TABLE 2. Brain morphometry.	
Brain Region	**Parameter Description and [Number]**
Frontal Cortex	Height
	Width
Dorsal Cortex	Thickness (1) on Level 3 at most dorsal point of external capsule, parallel to midsagittal line
	Thickness (2) on Level 3 along a line drawn at ~45°from the midsagittal plane
	Thickness on Level 4 along a line drawn at 90°to the surface and through the medial tip of the dentate gyrus
	Thickness on Level 5, measured in the same manner as 4A (immediately above)
Piriform Cortex	Thickness on Level 3 at midpoint between rhinal and amygdaloid fissures
	Thickness on Level 4 at midpoint between rhinal and amygdaloid fissures
	Thickness on Level 5 at midpoint between rhinal and amygdaloid fissures
Hippocampus	Length from midline to outer edge of most lateral pyramidal cells on Level 3
	Length from midline to outer edge of most lateral pyramidal cells on Level 4
	Width on Level 5 from inner zone of dentate gyrus to outer edge of CA2 [a]
	Dentate gyrus: Width on Level 4 at level of most medial part of lower limb of CA3 [a]
	Length on Level 4, measured parallel to a dorsal (horizontal) plane
	Width at widest point on Level 5
Corpus Callosum	Thickness at midline on Level 4
Thalamus	Height at midline on Level 4
	Width at widest point on Level 4
	Width at widest point on Level 5
Thalamus/Cortex	Overall width at the widest point of Level 4
Cerebellum	Height
	Length
	Preculminate Fissure: Thickness of molecular layer
	Thickness of outer granular layer [b]
	Thickness of inner granular layer
	Prepyramidal Fissure: Thickness of molecular layer
	Thickness of outer granular layer [b]
	Thickness of inner granular layer

Data taken from Appendix F, pp. 217-222, MRID 46239801.
[a] CA2 = Cornu Ammonis 2, and CA3 = Cornu Ammonis 3.
[b] Measured only in pups killed on PND 12; not found in adult rats.

On postnatal day 63, at least 10 animals/sex/group were deeply anesthetized via intraperitoneal sodium pentobarbitone and euthanized by perfusion fixation with formol saline at a volume approximately equivalent to their body weight. Brains were immediately

removed, whole brain and cerebellar weights were recorded, and the central and peripheral nervous tissues indicated below (X) were collected and preserved in an "appropriate" fixative. The brain tissues were processed in the following manner and examined. The cerebellum was cut sagittally at midline to make 2 blocks (levels 20 and 21), and the remainder of the brain (cerebrum and brain stem) was cut into 6 blocks by making transverse cuts at the following anatomic landmarks: the rostral edge of the olfactory bulb (level 1); the caudal edge of the olfactory bulb (level 2); the rostral edge of the median eminence (level 3); the caudal edge of the median eminence (level 5); the caudal edge of the cerebral hemispheres (level 6); and the midpoint of the remaining brain stem. The blocks were embedded in paraffin with the rostral or medial face down (as appropriate), sectioned, and stained with hematoxylin and eosin, and the spinal cord sections (including spinal nerve roots and dorsal root ganglia), eyes, and muscle sections were processed in the same manner. The peripheral nerve tissues were embedded in resin, sectioned in a "semi-thin" manner, and stained with toluidine blue. Detailed morphometric evaluations and enumeration of Purkinje cell bodies in lobule 8 of the cerebellum were conducted in the same manner as for pups killed on PND 12.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES** [transverse and longitudinal sections]
X	Cerebrum and brainstem (transverse sections)	X	Proximal sciatic nerve [a]
X	Cerebellum (sagittal sections)	X	Proximal tibial nerve [a]
		X	Distal tibial nerve (calf muscle branches) [a]
	SPINAL CORD [transverse and longitudinal sections]		**OTHER**
X	Cervical swelling	X	Eye (with optic nerve and retina) [a]
X	Lumbar swelling	X	Gastrocnemius muscle (transverse sections) [a]
		X	Spinal nerve roots at cervical swelling [b]
		X	Spinal nerve roots at lumbar swelling [b]
		X	Dorsal root ganglia at cervical swelling [b]
		X	Dorsal root ganglia at lumbar swelling [b]

Data taken from pp. 26-27, MRID 46239801.
[a] Right and left preserved; left processed for examination.
[b] Spinal nerve roots and dorsal root ganglia were included in transverse sections of the spinal cord.

In addition, at least 10 animals/sex/group were sacrificed on PND 63 by carbon dioxide exposure, and the brains from these animals were immediately removed, weighed (whole brain and removed cerebellum), and stored in an unspecified fixative.

D. DATA ANALYSIS:

1. **Statistical analyses:** Maternal body weight during gestation and during lactation were analyzed using analysis of covariance (ANCOVA) with GD 7 body weight and LD 1 body weight, respectively as covariants. Maternal body weight on LD 1 was analyzed using an analysis of variance (ANOVA).

   Offspring body weight was evaluated on a litter basis. ANCOVA was used to analyze the mean pup weight on PND 5 pre-cull and to analyze the mean weight of the selected F_1 offspring during PNDs 8-63. The mean body weight on PND 1 and on PND 5 post cull were respectively used as covariants, and both were analyzed used ANOVA.

   The following data were analyzed using ANOVA: gestation length; litter size; total litter weight on PNDs 1 and 5; motor activity measurements; maximum amplitude and time to maximum amplitude in startle response tests; (litter based) time to preputial separation or vaginal opening; (litter based) body weight at preputial separation or vaginal opening; brain morphometry data; and the number of Purkinje cell bodies per mm.

   Whole brain and cerebellum weights were analyzed using ANOVA and using ANCOVA with final body weight as the covariate. Brain to body weight ratio was not analyzed statistically.

   The following parameters were analyzed using Fisher's Exact Test: the proportion of litters with gestation length less than, equal to, and greater than 22 days; the proportion of whole litter loss in each group; and the proportion of males and females with observed developmental landmarks (preputial separation and vaginal opening) on each day.

   Data pertaining to live born pups, pup survival pre- and post-cull, and pup sex were evaluated as follows: 1) mean percentages were analyzed using ANOVA following the double arcsine transformation of Freeman and Tukey; 2) the proportion of pups born alive, the proportion of pups surviving, the proportion of litters with all pups born alive, the proportion of litters with all pups surviving and the proportion of male pups were analyzed using Fisher's Exact Test.

   Data from the water maze testing were analyzed as follows: 1) mean swimming times in the straight channel and for each individual trial in the Y-maze were analyzed using ANOVA; 2) mean percentages of successful trials at each cut-off value were analyzed using ANOVA following the double arcsine transformation of Freeman and Tukey.

   All statistical tests were two-sided and used significance levels of $p<0.05$ and $p<0.01$.

2. **Indices:**

a. **Reproductive indices:** No reproductive indices were calculated.

b. **Offspring viability indices:** No offspring viability indices were calculated. Proportions for live born and surviving pups were given in the results tables.

3. **Positive and historical control data:** Historical control data were provided for the incidences of minimal and slight demyelination of the proximal sciatic, proximal tibial, and distal tibial nerves and for limited brain morphometric measurements on PND 12 and 63. The demyelination data came from 10 studies conducted during October 2001 through April 2003. The brain morphometry data came from eight studies conducted during July 1995 through October 2002. No further information was provided concerning the materials, methods, and personnel used in those studies.

   No positive control data were provided. However, the following citations for previously conducted positive control and/or methodology validation studies were included in the "References" section of the study report (p. 36, MRID 46153302):

   - Allen, S. (1993) Measurement of motor activity in rat pups. CTL Report No. CTL/P/4155. MRID 44064701.
   - Allen, S. (1994) Assessment of learning and memory in rats. CTL Report No. CTL/P/4257. MRID 44064702.
   - Allen, S. (1995) Developmental neurotoxicity study in the rat using dietary restriction. CTL Report No. CTL/P/4383. MRID 44064705.
   - Allen, S. (1996) Trimethyltin chloride: investigation of neurotoxicity in rat pups using morphometrics and startle response. MRID 44064703.
   - Chivers, S. (2003) Motor activity: positive control study in rat pups. CTL Report No. CTL/WR0475/Validation/Report.
   - Milburn, G. (2003) Dizocilpine and mecamylamine: positive control water maze study in rats. CTL Report No. CTL/WR0442/Regulatory/Report.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** One treated female was sacrificed on LD 2 due to clinical signs of pale and piloerection. One control female failed to produce a litter and was killed on day 26. No abnormal FOB findings were recorded on any testing day.

2. **Body weight:** Selected group mean body weight data for pregnant or nursing dams are given in Table 3. Mean body weight and body weight gain of the treated dams were similar to those of controls throughout gestation and lactation.

TABLE 3. Maternal body weight (g) and body weight gain (g) during gestation and lactation[a]

Observations/study day	0 mg/kg/day	7.5 mg/kg/day
Gestation		
Mean body weight GD 1	255.7 ± 16.3	256.2 ± 17.3
Mean body weight GD 7	289.8 ± 16.8	290.7 ± 17.6
Mean body weight GD 14	324.5 ± 17.9	324.7 ± 18.8
Mean body weight GD 22	387.7 ± 25.1	401.8 ± 26.0
Weight gain GD 1-22[b]	132.0	145.6
Lactation		
Mean body weight LD 1	301.0 ± 24.3	304.9 ± 28.0
Mean body weight LD 7	309.4 ± 23.9	320.8 ± 24.6
Mean body weight LD 15	348.4 ± 25.3	356.5 ± 21.1
Mean body weight LD 22	362.7 ± 19.5	358.6 ± 20.3
Mean body weight LD 29	351.1 ± 18.1	341.7 ± 21.6

Data taken from Tables 3 and 4, pp. 69-71 and 72-73, respectively, MRID 46239801.
[a] Mean body weight values are given as Mean ± Standard Deviation.
[b] Calculated by reviewer using group mean body weight values; not analyzed statistically.

3. **Reproductive performance:** The reproductive performance of the parental females is summarized in Table 4. Pregnancy rate, gestation length, and number of live born litters were similar between the treated and control groups. One control dam had two stillborn pups.

TABLE 4. Reproductive performance.		
Observation	**0 mg/kg/day**	**7.5 mg/kg/day**
Number mated	30	30
Number pregnant (%)	30 (100%)	30 (100%)
Incidence of dystocia	0	0
Total litter resorptions	1	0
Litters born dead	1	0
Number of litters with live pups on LD 1	28	30
Mean (±SD) gestation duration (days)	22.0 ± 0.2	22.0 ± 0.0

Data taken from Table 5, p. 74 and Appendix 4, pp. 523-527, MRID 46239801.

4. **Maternal postmortem results:** Maternal necropsy was only conducted on animals that died intercurrently. The control animal that failed to produce a litter had 6 implantation sites in the uterus. Multiple adhesions of the abdominal organs were found in the treated dam that was sacrificed on LD 2.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) are summarized in Table 5. In the control group a total of five dams had complete litter loss and another eight litters had insufficient numbers of pups for selection of F_1 animals. Only two treated dams had complete litter loss. No treatment-related clinical signs of toxicity were observed in pups during lactation or post-weaning. Pups from the treated dam sacrificed with clinical signs on LD 2 showed hyperthermia prior to sacrifice.

TABLE 5. Litter size and viability		
Observation	**0 mg/kg/day**	**7.5 mg/kg/day**
Total number born	337	364
Number born alive	327	354
Number born dead	10	10
Total litter loss	5	2
Litters with insufficient pups[a]	8	3
Mean litter size		
LD 1	11.5 ± 3.7	11.6 ± 2.6
LD 5 (pre-cull)	9.5 ± 4.1	10.9 ± 2.6
LD 8	8.0 ± 0.0	7.9 ± 0.3
LD 15	6.9 ± 0.3	6.6 ± 0.8
LD 29	6.9 ± 0.3	6.5 ± 0.8
Sex Ratio (% male) on LD 1	50.4	54.6
Proportion born live (%)	94.6	97.7
Proportion surviving LDs 1-5 (%)	85.9	94.1

Data taken from Tables 6-10, pp. 75-79, respectively, and p. 523, MRID 46239801.
[a]At least 3 males and 3 females in a liter of at least 7 pups.

2. **Body weight:** Pre- and post-weaning offspring body weight data are summarized in Tables 6 and 7, respectively. No treatment-related effects on offspring body weight during or after lactation were observed. Body weight of the treated males and females was consistently greater than that of the controls throughout the study.

TABLE 6. Pre-weaning offspring body weight data (g)		
Parameter/Postnatal Day or Interval	**6 mg/kg/day**	**7.5 mg/kg/day**
Males		
Body weight PND 1	6.0 ± 0.7	6.2 ± 0.6
Body weight PND 5 (pre-cull)	9.3 ± 1.9	10.1 ± 0.9
Body weight PND 10	17.5 ± 2.0	20.1 ± 1.3
Body weight PND 14	27.1 ± 2.5	30.5 ± 1.5
Body weight PND 22	50.4 ± 4.0	54.8 ± 2.0
Body weight PND 29	90.9 ± 5.5	95.9 ± 2.9
BW gain PND 1-5 (pre-cull)[a]	3.3	3.9
BW gain PND 5 (post-cull) through PND 29[a]	81.6	85.8
Females		
Body weight PND 1	5.7 ± 0.8	5.8 ± 0.6
Body weight PND 5 (pre-cull)	9.0 ± 1.9	9.5 ± 1.0
Body weight PND 10	17.0 ± 1.9	18.9 ± 1.5
Body weight PND 14	26.6 ± 2.4	29.0 ± 2.1
Body weight PND 22	48.8 ± 3.5	52.3 ± 2.8
Body weight PND 29	84.6 ± 5.1	89.6 ± 3.4
BW gain PND 1-5 (pre-cull)[a]	3.3	3.7
BW gain PND 5 (post-cull) through PND 29[a]	75.7	80.1

Data taken from Tables 11 and 15, pp. 80 and 123-128, respectively, MRID 46239801.
[a]Calculated by reviewer from group mean values.

TABLE 7. Post-weaning offspring body weight data (g)		
Parameter/Postnatal Day or Interval	**0 mg/kg/day**	**7.5 mg/kg/day**
Males		
Body weight PND 29	90.9 ± 5.5	95.9 ± 2.9
Body weight PND 36	145.8 ± 9.1	153.2 ± 5.7
Body weight PND 50	258.6 ± 16.1	267.3 ± 8.8
Body weight PND 63	353.6 ± 19.2	358.9 ± 12.9
BW gain PND 29-63*	262.7	263.0
Females		
Body weight PND 29	84.6 ± 5.1	89.6 ± 3.4
Body weight PND 36	128.9 ± 7.1	135.3 ± 5.6
Body weight PND 50	188.7 ± 11.7	193.1 ± 7.5
Body weight PND 63	223.3 ± 14.8	224.8 ± 8.9
BW gain PND 29-63*	138.7	135.2

Data taken from Table 15, pp. 123-128, MRID 46239801.
*Calculated by reviewer from group mean values.

3. **Developmental landmarks:**

a) **Sexual maturation:** Age and body weight at sexual maturation are given in Table 8. Age at attainment for the treated animals was significantly earlier than that of the control animals and corresponded with slightly higher body weight for the treated group.

TABLE 8. Mean (±SD) age and body weight at sexual maturation		
Parameter	**0 mg/kg/day**	**7.5 mg/kg/day**
N (M/F)	16/16	24/24
Males		
Preputial separation (days)	44.4 ± 1.0	43.7* ± 0.9
Body wt. at attainment (g)	211.6 ± 13.1	215.1 ± 6.8
Females		
Vaginal opening (days)	37.5 ± 2.2	36.4* ± 1.1
Body wt. at attainment (g)	135.2 ± 10.0	136.4 ± 8.0

Data obtained from Table 16, pp. 129-130, MRID 46239801.
Significantly different from control: *p ≤ 0.05.

b) **Developmental landmarks:** Other developmental landmarks, such as eye opening, incisor eruption, pinna unfolding, and fur growth, were not monitored.

4. **Behavioral assessments:**

a) **Functional observational battery:** No abnormal findings were reported for any animal on any testing day.

b) **Motor activity:** Mean total motor activity counts are reported in Table 9. Activity generally increased with increasing age, and no significant differences were found between the total activity counts of the treated and control groups of either sex on any testing day. Statistically significant differences were noted sporadically for individual sub-sessions, but no dose- or time-related pattern was evident. No habituation was seen in either sexes on all testing day. However, sub-session counts were somewhat variable between successive intervals.

TABLE 9. Motor activity data: total activity counts for session

Test Day	0 mg/kg/day	7.5 mg/kg/day
Males		
PND 14	37.9 ± 17.9	77.0 ± 75.1
PND 18	158.3 ± 129.8	246.8 ± 137.3
PND 22	303.1 ± 223.0	288.6 ± 124.2
PND 60	440.9 ± 112.7	544.8 ± 150.9
Females		
PND 14	144.8 ± 103.7	113.6 ± 63.5
PND 18	274.1 ± 186.3	188.2 ± 112.3
PND 22	402.6 ± 188.1	280.2 ± 166.3
PND 60	579.1 ± 83.5	578.2 ± 71.2

Data taken from Table 17, pp. 131-138, MRID 46239801.
N = 8/sex for control and 11-12/sex for treated animals

c) **Auditory startle reflex habituation:** Results of the auditory startle reflex habituation testing are given in Table 10 (maximum startle amplitude) and Table 11 (time to maximum amplitude). No treatment-related differences were observed on either day. Habituation was seen over successive trial blocks in all groups on both days.

TABLE 10. Auditory startle reflex habituation: maximum amplitude (Vmax)			
Test Day	**Block**	**0 mg/kg/day**	**7.5 mg/kg/day**
Males			
PND 23	1-10	381.1 ± 76.1	396.5 ± 128.8
	11-20	288.1 ± 68.1	327.2 ± 73.0
	21-30	275.8 ± 37.9	282.4 ± 74.8
	31-40	248.0 ± 56.6	266.5 ± 56.6
	41-50	217.7 ± 54.1	251.1 ± 80.4
PND 61	1-10	1962.2 ± 1001.9	1424.1 ± 528.2
	11-20	1570.4 ± 411.1	1121.8 ± 749.8
	21-30	1193.9 ± 423.7	953.8 ± 334.8
	31-40	1123.9 ± 322.0	879.9 ± 390.3
	41-50	1106.1 ± 211.3	853.7 ± 372.9
Females			
PND 23	1-10	304.2 ± 50.2	352.5 ± 106.7
	11-20	236.5 ± 61.5	242.7 ± 66.4
	21-30	209.7 ± 81.1	233.6 ± 57.6
	31-40	194.2 ± 77.0	210.6 ± 67.5
	41-50	199.0 ± 67.9	199.2 ± 39.3
PND 61	1-10	912.5 ± 186.6	941.7 ± 428.9
	11-20	734.7 ± 173.4	920.8 ± 338.1
	21-30	593.7 ± 347.9	778.7 ± 219.7
	31-40	547.2 ± 190.1	802.8* ± 261.5
	41-50	632.8 ± 283.0	642.3 ± 222.9

Data taken from Table 18, pp. 139-142, MRID 46239801.
Significantly different from control: * $p<0.05$

TABLE 11. Auditory startle reflex habituation: time to maximum amplitude (ms)			
Test Day	**Block**	**0 mg/kg/day**	**7.5 mg/kg/day**
Males			
PND 23	1-10	25.4 ± 4.4	25.2 ± 5.9
	11-20	19.2 ± 0.6	20.5 ± 2.7
	21-30	19.8 ± 1.4	20.5 ± 1.7
	31-40	20.3 ± 1.6	20.6 ± 2.4
	41-50	20.4 ± 2.2	20.2 ± 1.6
PND 61	1-10	28.9 ± 16.7	25.5 ± 5.8
	11-20	23.6 ± 6.2	23.4 ± 4.8
	21-30	23.5 ± 2.8	24.1 ± 4.5
	31-40	23.6 ± 2.7	25.4 ± 4.7
	41-50	23.6 ± 2.9	25.4 ± 4.2
Females			
PND 23	1-10	25.2 ± 5.2	25.9 ± 10.6
	11-20	19.9 ± 1.1	22.0** ± 1.7
	21-30	21.6 ± 5.2	20.7 ± 2.0
	31-40	20.2 ± 1.2	21.5 ± 3.5
	41-50	19.6 ± 0.7	21.6 ± 3.3
PND 61	1-10	22.6 ± 2.1	25.0 ± 3.5
	11-20	23.0 ± 2.0	22.6 ± 4.1
	21-30	25.1 ± 2.3	23.9 ± 3.9
	31-40	25.4 ± 3.4	23.8 ± 3.9
	41-50	24.8 ± 3.4	25.2 ± 5.8

Data taken from Table 19, pp. 143-146, MRID 46239801
Significantly different from control: * *p<0.01

d) **Learning and memory testing:** Selected data from the water maze testing are given in Table 12 for PNDs 24/27 and Table 13 for PNDs 59/62. No treatment-related changes in learning or memory were observed on either sex. The proportion of successful trials at a specified cut-off criteria was not affected by treatment at either testing interval. On the first day, learning was evident in each group at both time points as a decrease in mean swim time for Trial 6 compared to the mean swim time for Trial 1. Memory was evident in all groups at both time points as a decrease in the Trial 1 swim time on the second day of testing compared to the Trial 1 swim time on the first day of testing. On PND 24,

treated males had a significantly faster Trial 4 swim time compared to the controls and on PND 62 females had a significantly slower Trial 6 swim time compared to the controls. However, straight channel swim times were similar between the treated and control groups on all testing days.

TABLE 12. Selected water maze performance parameters for offspring at postnatal days 24 and 27.

Session/Parameter		0 mg/kg/day	7.5 mg/kg/day
Males			
PND 24	Swim time (seconds):		
	Trial 1	10.45 ± 5.62	11.66 ± 7.42
	Trial 6	5.66 ± 3.07	4.37 ± 1.72
	% Successful Trials: [a]		
	Cut-off time = 3 sec	6.3 ± 8.3	8.0 ± 13.2
	Cut-off time = 5 sec	45.8 ± 30.1	59.4 ± 22.4
	Cut-off time = 10 sec	81.3 ± 27.1	84.1 ± 14.6
PND 27	Swim time (seconds):		
	Trial 1	7.36 ± 3.69	7.96 ± 5.48
	Trial 6	4.65 ± 2.39	5.11 ± 4.02
	% Successful Trials: [a]		
	Cut-off time = 3 sec	26.0 ± 28.5	22.5 ± 22.3
	Cut-off time = 5 sec	63.5 ± 23.7	65.9 ± 27.3
	Cut-off time = 10 sec	93.8 ± 8.3	89.1 ± 15.6
Females			
PND 24	Swim time (seconds):		
	Trial 1	11.02 ± 5.85	9.41 ± 3.98
	Trial 6	4.07 ± 1.51	4.45 ± 2.91
	% Successful Trials: [a]		
	Cut-off time = 3 sec	17.7 ± 23.1	13.6 ± 17.5
	Cut-off time = 5 sec	56.3 ± 24.2	53.0 ± 22.8
	Cut-off time = 10 sec	87.5 ± 14.3	80.3 ± 17.5
PND 27	Swim time (seconds):		
	Trial 1	8.23 ± 5.38	6.41 ± 4.48
	Trial 6	4.32 ± 2.97	4.33 ± 2.28
	% Successful Trials: [a]		
	Cut-off time = 3 sec	34.4 ± 27.5	30.3 ± 29.8
	Cut-off time = 5 sec	76.0 ± 15.6	70.5 ± 18.5
	Cut-off time = 10 sec	90.6 ± 12.1	90.2 ± 11.1

Data taken from Tables 20 and 21, pp. 147-150 and 155-161, respectively, MRID 46239801.
N = 16/sex for control and 22-23/sex for treated animals.
[a]A successful trial is one that is completed in less than the given cut-off time. The percentage of trials meeting a specific criterion was calculated for each individual animal and used to determine the group mean for that criterion.

TABLE 13. Selected water maze performance parameters for offspring at postnatal days 59 and 62.			
Session/Parameter		0 mg/kg/day	7.5 mg/kg/day
Males			
PND 59	Swim time (seconds):		
	Trial 1	13.63 ± 4.82	12.63 ± 5.43
	Trial 6	4.20 ± 2.06	4.85 ± 2.78
	% Successful Trials: [a]		
	Cut-off time = 3 sec	14.6 ± 18.1	17.4 ± 22.7
	Cut-off time = 5 sec	50.0 ± 21.9	50.0 ± 27.5
	Cut-off time = 10 sec	83.3 ± 10.5	82.6 ± 11.8
PND 62	Swim time (seconds):		
	Trial 1	5.03 ± 2.86	4.95 ± 2.11
	Trial 6	6.37 ± 3.37	4.55 ± 2.49
	% Successful Trials: [a]		
	Cut-off time = 3 sec	22.9 ± 18.1	26.8 ± 30.0
	Cut-off time = 5 sec	56.3 ± 25.0	60.9 ± 24.4
	Cut-off time = 10 sec	89.6 ± 18.1	91.3 ± 13.2
Females			
PND 59	Swim time (seconds):		
	Trial 1	12.99 ± 5.34	13.61 ± 6.16
	Trial 6	4.74 ± 2.79	4.26 ± 3.79
	% Successful Trials: [a]		
	Cut-off time = 3 sec	14.4 ± 15.3	18.1 ± 18.7
	Cut-off time = 5 sec	53.3 ± 16.9	56.5 ± 21.8
	Cut-off time = 10 sec	83.3 ± 14.1	81.2 ± 11.6
PND 62	Swim time (seconds):		
	Trial 1	4.83 ± 2.24	4.21 ± 2.15
	Trial 6	4.44 ± 2.60	9.21* ± 6.65
	% Successful Trials: [a]		
	Cut-off time = 3 sec	26.7 ± 25.8	34.8 ± 27.5
	Cut-off time = 5 sec	63.3 ± 22.9	57.2 ± 22.4
	Cut-off time = 10 sec	88.9 ± 12.1	76.1 ± 21.2

Data taken from Tables 20 and 21, pp. 151-154 and 163-169, respectively, MRID 46239801.
N = 16/sex for control and 22-23/sex for treated animals.
[a]A successful trial is one that is completed in less than the given cut-off time. The percentage of trials meeting a specific criterion was calculated for each individual animal and used to determine the group mean for that criterion.
Significantly different from control: *p ≤ 0.05.

5. Postmortem results:

a) Brain weight: Brain weight data are given in Table 14. No treatment-related effects on whole brain or cerebellum weights were observed at either time point. On PND 12, the absolute weight of the cerebellum from treated females was significantly greater than that of the controls.

TABLE 14. Brain weight data.		
Study Day/Parameter	**0 mg/kg/day**	**7.5 mg/kg/day**
Males		
PND 12:		
Terminal body weight (g)	21.6 ± 1.7	24.1 ± 1.9
Brain weight (g)	1.03 ± 0.09	1.06 ± 0.12
Brain/BW ratio (%)	4.76 ± 0.40	4.41 ± 0.51
Cerebellum weight (g)	0.120 ± 0.013	0.123 ± 0.007
Cerebellum/BW ratio (%)	0.561 ± 0.088	0.513 ± 0.048
PND 63 (post perfusion):		
Terminal body weight (g)	363.5 ± 26.8	360.7 ± 22.8
Brain weight (g)	1.93 ± 0.22	1.81 ± 0.15
Brain/BW ratio (%)	0.53 ± 0.04	0.50 ± 0.07
Cerebellum weight (g)	0.311 ± 0.028	0.292 ± 0.044
Cerebellum/BW ratio (%)	0.086 ± 0.006	0.081 ± 0.011
Females		
PND 12:		
Terminal body weight (g)	20.4 ± 2.6	23.7 ± 1.8
Brain weight (g)	1.01 ± 0.09	1.01 ± 0.09
Brain/BW ratio (%)	5.01 ± 0.59	4.30 ± 0.54
Cerebellum weight (g)	0.105 ± 0.015	0.120* ±0.006
Cerebellum/BW ratio (%)	0.517 ± 0.084	0.509 ± 0.053
PND 63 (post perfusion):		
Terminal body weight (g)	231.9 ± 16.7	225.3 ± 13.7
Brain weight (g)	1.76 ± 0.16	1.69 ± 0.14
Brain/BW ratio (%)	0.76 ± 0.10	0.75 ± 0.07
Cerebellum weight (g)	0.272 ± 0.032	0.267 ± 0.016
Cerebellum/BW ratio (%)	0.118 ± 0.018	0.119 ± 0.009

Data taken from Table 23, pp. 173-179, MRID 46239801.
N = 8-12/sex/group
Significantly different from control: *$p < 0.05$.

b) **Macroscopic examination:** Offspring were not subjected to gross examination.

c) **Neurohistopathology:** No treatment-related effects were seen at PND 12 or 63. On PND 12, hemorrhage in the brain was found in one male in each of the control and treated groups. At PND 63, minimal to slight demyelination of the distal tibial, proximal sciatic, and proximal tibial nerves was observed in several animals from all groups. The incidences of the peripheral nerve findings were within the provided historical control ranges.

d) **Morphometric evaluation**: Morphometric measurements taken in the cerebrum and brain stem are given in Tables 15 and 16 for males and females, respectively, and those taken in the cerebellum are given in Table 17. Evaluation/interpretation of the morphometric data was confounded due to the low viability of control PND 12 animals. However, statistical significance was attained for some measurements, but these were sporadic, not consistent over time or sex, and not consistent within a region.

The number of Purkinje cells was similar between the treated and control groups.

TABLE 15. Brain morphometry of cerebrum and brainstem in male offspring (mm).				
Region/Section	**0 mg/kg/day**	**7.5 mg/kg/day**	**0 mg/kg/day**	**7.5 mg/kg/day**
	PND 12 [N = 8 and 12]		**PND 63 [N = 11 and 12]**	
Frontal Cortex:				
Height - Level 2	5.36 ± 0.15	5.35±0.36	6.86 ± 0.19	6.95 ± 0.35
Width - Level 2	4.38 ± 0.12	4.36 ± 0.32	5.14 ± 0.40	5.40 ± 0.32
Dorsal Cortex:				
Thickness (1) - Level 3	1.18 ± 0.13	1.25 ± 0.09	1.22 ± 0.11	1.15 ± 0.08
Thickness (2) - Level 3	1.24 ± 0.09	1.33 ± 0.16	1.56 ± 0.22	1.52 ± 0.11
Thickness - Level 4	1.11 ± 0.12	1.14 ± 0.12	1.11 ± 0.17	1.19 ± 0.11
Thickness - Level 5	1.02 ± 0.05	1.07 ± 0.06	1.25 ± 0.14	1.29 ± 0.13
Piriform Cortex:				
Thickness - Level 3	1.11 ± 0.06	1.05 ± 0.11	1.21 ± 0.07	1.19 ± 0.12
Thickness - Level 4	1.08 ± 0.05	1.04 ± 0.10	1.06 ± 0.06	1.15** ± 0.09
Thickness - Level 5	1.02 ± 0.07	0.98 ± 0.08	1.08 ± 0.04	1.08 ± 0.10
Hippocampus				
Length - Level 3	3.28 ± 0.25	3.00 ± 0.38	2.47 ± 0.27	2.41 ± 0.22
Length - Level 4	4.32 ± 0.27	3.88* ± 0.42	3.63 ± 0.46	3.48 ± 0.46
Width - Level 5	1.31 ± 0.08	1.18* ± 0.16	1.31 ± 0.11	1.31 ± 0.15
Dentate gyrus length - Level 4	1.54 ± 0.16	1.45 ± 0.13	1.66 ± 0.19	1.61 ± 0.18
Dentate gyrus width - Level 4	0.46 ± 0.04	0.50 ± 0.08	0.57 ± 0.04	0.58 ± 0.06
Dentate gyrus width - Level 5	0.69 ± 0.07	0.62 ± 0.12	0.64 ± 0.15	0.68 ± 0.18
Corpus Callosum				
Thickness - Level 4	0.67 ± 0.12	0.61 ± 0.13	0.36 ± 0.05	0.38 ± 0.05
Thalamus:				
Height - Level 4	5.53 ± 0.29	5.41 ± 0.29	5.37 ± 0.23	5.20 ± 0.28
Width - Level 4	8.35 ± 0.57	8.14 ± 0.52	8.69 ± 0.35	8.82 ± 0.58
Width - Level 5	7.53 ± 0.55	6.90* ± 0.52	7.70 ± 0.34	7.72 ± 0.25
Thalamus/Cortex				
Overall width - Level 4	13.53 ± 0.86	13.16 ± 0.50	14.16 ± 1.13	14.47 ± 0.48

Data taken from Table 24, pp. 180-203, MRID 46239801.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

TABLE 16. Brain morphometry of cerebrum and brainstem in female offspring (mm).				
Region/Section	**0 mg/kg bw/day**	**7.5 mg/kg/day**	**0 mg/kg/day**	**7.5 mg/kg/day**
	PND 12 [N = 8 and 10]		**PND 63 [N = 11 and 12]**	
Frontal Cortex:				
Height - Level 2	5.26 ± 0.31	5.55 ± 0.32	6.80 ± 0.36	6.93 ± 0.32
Width - Level 2	4.25 ± 0.21	4.44 ± 0.25	5.23 ± 0.27	5.32 ± 0.36
Dorsal Cortex:				
Thickness - Level 3	1.10 ± 0.12	1.23** ± 0.06	1.23 ± 0.08	1.20 ± 0.10
Thickness - Level 3	1.20 ± 0.10	1.35* ± 0.12	1.55 ± 0.20	1.51 ± 0.12
Thickness - Level 4	1.06 ± 0.06	1.14* ± 0.09	1.21 ± 0.14	1.20 ± 0.08
Thickness - Level 5	1.07 ± 0.03	1.10 ± 0.09	1.19 ± 0.09	1.22 ± 0.11
Piriform Cortex:				
Thickness - Level 3	1.12 ± 0.08	1.06 ± 0.15	1.19 ± 0.08	1.20 ± 0.13
Thickness - Level 4	1.08 ± 0.07	1.07 ± 0.10	1.20 ± 0.07	1.13* ± 0.07
Thickness - Level 5	0.99 ± 0.06	1.05 ± 0.09	1.09 ± 0.07	1.09 ± 0.10
Hippocampus:				
Length - Level 3	3.05 ± 0.33	3.14 ± 0.13	2.65 ± 0.34	2.54 ± 0.34
Length - Level 4	4.23 ± 0.15	3.88* ± 0.34	3.81 ± 0.40	3.75 ± 0.31
Width - Level 5	1.21 ± 0.14	1.35 ± 0.07	1.34 ± 0.06	1.36 ± 0.07
Dentate gyrus length - Level 4	1.47 ± 0.18	1.45 ± 0.09	1.75 ± 0.16	1.74 ± 0.17
Dentate gyrus width - Level 4	0.45 ± 0.04	0.46 ± 0.06	0.57 ± 0.02	0.58 ± 0.05
Dentate gyrus width - Level 5	0.59 ± 0.06	0.69* ± 0.09	0.59 ± 0.05	0.61 ± 0.08
Corpus Callosum:				
Thickness - Level 4	0.65 ± 0.09	0.58 ± 0.13	0.42 ± 0.05	0.39 ± 0.07
Thalamus:				
Height - Level 4	5.32 ± 0.20	5.26 ± 0.23	5.42 ± 0.22	5.32 ± 0.29
Width - Level 4	8.30 ± 0.20	7.86* ± 0.43	8.46 ± 0.40	8.45 ± 0.30
Width - Level 5	6.93 ± 0.23	7.05 ± 0.39	7.51 ± 0.25	7.54 ± 0.25
Thalamus/Cortex				
Overall width - Level 4	13.12 ± 0.36	12.74 ± 0.53	14.10 ± 0.47	14.08 ± 0.44

Data taken from Table 24, pp. 180-203, MRID 46239801.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

TABLE 17. Brain morphometry of cerebellum				
Parameter Description	0 mg/kg bw/day	7.5 mg/kg/day	0 mg/kg/day	7.5 mg/kg/day
	Males		Females	
PND 12 [N = 8-12]				
Height (mm)	3.74 ± 0.16	3.56* ± 0.17	3.38 ± 0.37	3.72 ± 0.19
Length (mm)	4.32 ± 0.31	4.09 ± 0.35	3.98 ± 0.37	3.89 ± 0.30
Thickness of cerebellar cortex layers				
Preculminate Fissure:				
Molecular layer (mm)	75.9 ± 9.0	70.9 ± 6.0	69.2 ± 6.9	69.8 ± 6.4
Outer granular layer (mm)	39.1 ± 6.6	38.2 ± 8.9	35.5 ± 4.4	38.7 ± 7.7
Inner granular layer (μm)	151 ± 24	150 ± 27	141 ± 20	132 ± 22
Prepyramidal Fissure:				
Molecular layer (mm)	65.0 ± 10.2	57.7 ± 6.3	56.4 ± 8.8	57.2 ± 8.2
Outer granular layer (μm)	44.6 ± 3.5	49.3 ± 9.8	44.8 ± 7.0	48.5 ± 9.5
Inner granular layer (mm)	137 ± 22	142 ± 22	123 ± 9	138* ± 16
PND 63				
Height (mm)	5.52 ± 0.57	5.23 ± 0.23	5.15 ± 0.21	4.93 ± 0.28
Length (mm)	6.29 ± 0.72	6.81 ± 0.46	6.70 ± 0.25	6.37 ± 0.53
Thickness of cerebellar cortex layers				
Preculminate Fissure:				
Molecular layer (mm)	99.4 ± 12.0	101.0 ± 9.8	105.0 ± 12.0	104.1 ± 6.6
Inner granular layer (μm)	80 ± 11	76 ± 16	91 ± 8	86 ± 7
Prepyramidal Fissure:				
Molecular layer (mm)	119.4 ± 20.0	117.3 ± 19.1	113.9 ± 10.5	108.9 ± 13.0
Inner granular layer (mm)	73 ± 10	81* ± 7	77 ± 10	77 ± 10

Data taken from Table 24, pp. 180-203, MRID 46239801.
Significantly different from control: * p<0.05.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that there were no treatment-related effects on the F_0 parent females. The study author also concluded that no evidence of toxicity, including neurotoxicity, was seen in the F_1 offspring. Poor survival of pups from the group dosed with 7.5 mg/kg/day in a previous study was not repeated in the current study.

B. REVIEWER COMMENTS:

The current study was designed as a supplement to a definitive developmental neurotoxicity study (MRID 46153302). Only one dose level was used in an attempt to confirm findings in the previous study.

The reviewer agrees that no clear evidence of maternal toxicity was observed. **Therefore, the maternal systemic and neurotoxicity LOAEL is not identified, and the NOAEL is greater than or equal to 7.5 mg/kg bw/day.**

Excessive litter losses in the control group during lactation reduced the number of litters available for assignment of offspring to further testing. The reason for the pup mortality is unknown but was also observed in the definitive developmental neurotoxicity study (MRID 46153302) at the same dose used in the current study. Therefore, pup mortality is not related to treatment with the test article, but may reflect a problem with the animals or with the testing facility.

No evidence for offspring toxicity was observed. Pups from the treated group actually had greater body weight than those of the control group. Correspondingly, the higher body weight of the treated animals resulted in earlier attainment of sexual maturation.

Developmental neurotoxicity was not seen in the offspring as measured by the FOB, motor activity, auditory startle reflex habituation, or learning and memory tests. Evaluation/ interpretation of the morphometric data was confounded due to the low viability of control PND 12 animals.

The offspring systemic and neurotoxicity NOAEL is 7.5 mg/kg/day (HDT). The offspring LOAEL is not established.

C. STUDY DEFICIENCIES:

1) The high pup mortality in controls during lactation obscures the interpretation of the study results, specially, with regard to the mortality seen at the high dose group.

2) The number of available litters for F1 offspring selection at the high dose group was inadequate for evaluation.