UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

September 28, 2005

MEMORANDUM

Subject: **TERBUFOS: REVIEW OF DEVELOPMENTAL NEUROTOXICITY STUDY**

PC CODE: 105001
DP BARCODE: D300180
TXR#: 0052438

To: Robert McNally
Reregistration Branch I
Special Review and Reregistration Division (7508C)

From: Paul Chin, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

Through: Whang Phang, Ph.D.
Senior Scientist
Reregistration Branch I
Health Effects Division (7509C)

The registrant, BASF Corporation, submitted a Developmental Neurotoxicity Study in Rats. This study was reviewed by the contractor, Oak Ridge National Laboratory and went through the secondary review process in HED. The DER for this study is attached to this memorandum. The citation and the results of the study are summarized as follows:

CITATION: Kaufmann, W., Schneider, S., Deckardt, K, and van Ravenzwaay. (2004) BAS 316 I (terbufos) - Developmental Neurotoxicity Study in Wistar Rats Oral Administration to the Dams and Pups (Gavage). Experimental Toxicology and Ecology,.BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Laboratory Project I.D. 66R0090/02012; February 20, 2004. MRID 46214301. Unpublished

In the developmental neurotoxicity study of terbufos in rats, there were no treatment-related

effects on mortality, clinical signs, body weight, or food consumption in dams. However, high-dose dams (0.15 mg/kg/day) exhibited toxicologically significant inhibition in erythrocyte and brain cholinesterase activity on postnatal day 21.

In offspring, there were no treatment-related deaths, clinical signs or effects on birth weight, developmental landmarks, FOB parameters, auditory startle reflex, learning and memory, brain weight, brain morphology, or neuropathology. However, there were treatment-related increases in motor activity in the mid (0.08 mg/kg/day) and high (0.15 mg/kg/day) dose groups. In addition, significant dose-related inhibition of serum, erythrocyte, and brain ChE activity were observed in these dose groups.

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

DATA EVALUATION RECORD

TERBUFOS

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 46214301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 32-2004

Primary Reviewer:
Cheryl B. Bast, Ph.D., D.A.B.T.

Signature: Cheryl B Bast
Date: JUL 13 2004

Secondary Reviewers:
Carol Wood, Ph.D., D.A.B.T.

Signature: Carol S Wood
Date: JUL 13 2004

Robert H. Ross, M.S. Group Leader

Signature: Robert H. Ross
Date: JUL 13 2004

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 13 2004

Disclaimer

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

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EPA Reviewer: Paul Chin, Ph.D. **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509C) **Date** 9/27/05
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/27/05
EPA Secondary Reviewer: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 10/11/05

TXR#: 0052438

DATA EVALUATION RECORD

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

PC CODE: 105001

DP BARCODE: D300180
SUBMISSION NO.: NA

TEST MATERIAL (PURITY): Terbufos (88.88%)

SYNONYMS: BAS 361 I

CITATION: Kaufmann, W., Schneider, S., Deckardt, K, and van Ravenzwaay. (2004) BAS 316 I (terbufos) - Developmental Neurotoxicity Study in Wistar Rats Oral Administration to the Dams and Pups (Gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen. Germany. Laboratory Project I.D. 66R0090/02012; February 20, 2004. MRID 46214301. Unpublished

SPONSOR: BASF Corporation, Agricultural Products Division, Research Triangle Park, NC.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46214301), Terbufos (88.88% a.i., batch # AC 12251-100) was administered in corn oil to 42 pre-mated female Wistar (CrlGlxBrlHan:WI) rats/dose by gavage at doses of 0, 0.01, 0.08 and 0.15 mg/kg/day from gestation day (GD) 6 through postnatal day (PND) 10 in a volume of 5 mL/kg body weight. The test material was administered to offspring at the same doses from post-natal days 11 through 21. A Functional Observational Battery (FOB) was performed on 10 dams/dose on gestation days 7 and 14, and on 10 dams/dose on lactation days 7 and 14. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) on PND 4, 11, 21, 35, 45, and 60; assessment of motor activity on PND 13, 17, 21, and 60; auditory startle response habituation on PND 24 and 60; learning and memory on PND 23 and 60. On postnatal day 22 and 60, the whole brain was collected from 10 pups/sex/dose level for micropathologic examination and morphometric analysis. Brain, erythrocyte, and serum cholinesterase activities were measured in offspring (10/dose group) on days 4 and 21 and in dams (10/dose group) on postnatal day 21. Pup physical development was assessed by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

In dams, there were no treatment-related effects on mortality, clinical signs, body weight, or food consumption. No treatment-related effects on reproductive parameters were observed. High-dose dams exhibited toxicologically significant inhibition in erythrocyte (26% inhibition; $p \leq 0.01$) and brain (33% inhibition; $p \leq 0.01$) cholinesterase activity on PND 21. Erythrocyte cholinesterase activity was inhibited 13% ($p \leq 0.05$) in mid-dose dams; however, this effect, while treatment-related, is not considered toxicologically significant due to the small magnitude of change. No other cholinesterase activity effects were noted in dams on PND 21.

The maternal LOAEL was 0.15 mg/kg/day based on inhibition of erythrocyte and brain cholinesterase activity. The maternal NOAEL was 0.08 mg/kg/day

In offspring, there were no treatment-related deaths, clinical signs or effects on birth weight, developmental landmarks, FOB parameters, auditory startle reflex, learning and memory, brain weight, brain morphology, or neuropathology. At the high dose (0.15 mg/kg/day), male and female offspring body weight was approximately 4-6% lower from LD 11 through weaning at day 21. Body weight gain was decreased for high-dose males and females during lactation days 1-11 (up to 11% decrease) and lactation days 12-13 (up to 14% decrease). No body weight effects were noted for low- or mid-dose pups during lactation. There were no toxicologically-significant post-weaning body weight effects.
There were treatment-related increases in motor activity at the mid and high dose groups. On PND 13, there were increases in males at the mid (35%) and high (58%) dose groups and in females at the mid (34%) and high (19%) dose groups. On PND 17 there were increases in males at the mid (18%) and high (26%) dose groups and in females at the mid (15%) and high (64%) dose groups.

No treatment-related changes were noted in serum, erythrocyte or brain cholinesterase activity for male or female pups on PND 4. On PND 21, significant dose-related inhibition ($p \leq 0.05$ or 0.01) of serum (56-86% decrease), erythrocyte (47-84% decrease), and brain (38-75% decrease) ChE activity were observed in mid- and high-dose males and females. No treatment-related effects were noted for low-dose animals.

The offspring LOAEL was 0.08 based on increased motor activity in males and females on PND 13 and 17 and inhibition of serum, erythrocyte, brain cholinesterase activity in both sexes. The NOAEL was 0.01 mg/kg/day.

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

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Terbufos

Description:	Liquid/colorless to pale yellow
Batch #:	AC 12251-100
Purity:	88.88 % a.i.
Compound Stability:	"Proven by reanalysis"
CAS # of TGAI:	13071-79-9

2. Vehicle: corn oil

3. Test animals (P):

Species:	Rat
Strain:	Wistar (CrlGlxBrlHan:WI)
Age at study initiation:	Time-mated females: 10-12 wks
Wt. at study initiation:	140.9-195.0 g (on post-coital day 0)
Source:	Charles River Laboratories, Germany
Housing:	Individually in stainless steel cages, except from gestation day 18 to lactation day 21 when dams, litters and subset II were in Makrolon type M III cages
Diet:	ground or pelleted Kliba maintenance diet rat/mouse/hamster (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 20-24°C **Humidity:** 30-70% **Air changes:** Not provided **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	9 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: September 9, 2002; End: January 23, 2004

2. **Study schedule:** Time-mated female Wistar rats (42/dose group; 41 control) were administered the test material by gavage from gestation day (GD) 6 through postnatal day (PND) 10. On postnatal day 4, litters were standardized to 8 pups, sexes were represented as equally as possible. Pups were weaned from the dam on PND 21; dams were sacrificed after the weaning. The test material was administered by gavage to pups from PND 11 through PND 21. Pups remained on study up to PND 62.

3. **Mating procedure:** Time mating of females was carried out at Charles River Laboratories, Germany. The day that a vaginal plug or sperm in a vaginal smear was detected was designated gestation day (GD) 0. Females presumed to be pregnant were delivered to the testing laboratory on GD 0.

4. **Animal assignment:** The mated females were randomly assigned to treatment groups upon arrival at the testing laboratory, as shown in Table 1. The method used for assigning animals

was not stated. On GDs 7 and 14 and lactation days (LD) 7 and 14, the females were examined outside the cage using a functional observation battery (FOB) of tests.

Eight subsets of 10 pups/sex/group were assigned for detailed clinical observations (FOB), motor activity, auditory startle, learning and memory, cholinesterase and neuropathology as noted in Table 1.

TABLE 1. Study design

Experimental parameter	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	41	42	42	42
FOB (GDs 7 and 14, LDs 7 and 14)	10	10	10	10
Offspring				
	No. of offspring assigned			
Subset I - Immersion fixation, brain preservation (PND 11)	10/sex	10/sex	10/sex	10/sex
Subset II - Perfusion fixation, brain weight and neuropathology (PND 22)	10/sex	10/sex	10/sex	10/sex
Subset III - Auditory startle test (LDs 24, 60), perfusion fixation, brain weights, neuropathology (PND 62)	10/sex	10/sex	10/sex	10/sex
Subset IV - FOB (LDs 4, 11, 21, 35, 45, 60), motor activity (PNDs 13, 17, 21, 60)	10/sex	10/sex	10/sex	10/sex
Subset V - Learning and memory test (water maze test) (PND 23)	10/sex	10/sex	10/sex	10/sex
Subset VI - Learning and memory test (water maze test) (PND 60)	10/sex	10/sex	10/sex	10/sex
Subset VII - Cholinesterase measurements (PND 4)	10/sex	10/sex	10/sex	10/sex
Subset VIII - Cholinesterase measurements (PND 21)	10/sex	10/sex	10/sex	10/sex

Data obtained from pages 30 and 36. MRID 46214301

5. **Dose selection rationale:** The low-dose level was expected to be a NOAEL. No other dose selection rationale was provided.

6. **Dosage administration:** Terbufos was administered to maternal animals by gavage on GD 6 through lactation day 10, in a volume of 5 mL/kg of body weight. Dosing was based on the most recent body weight determination. The test material was administered to pups by gavage at the same doses used for dams from PNDs 11 through 21.

7. **Dosage preparation and analysis:** Formulations were prepared on the day of administration by mixing appropriate amounts of test substance with corn oil. Prior to the start of the study, stability of the test substance in corn oil was evaluated for a period of at least 7 days at room temperature. Homogeneity (top, middle, and bottom) was not evaluated.

During the study, samples of the oily test substance solutions were analyzed by HPLC three times for concentration.

Results:

Homogeneity analysis: Homogeneity was not determined since the test substance preparations were true solutions.

Stability analysis: The test material was stable in corn oil at room temperature for 7 days (91.9% of initial value at day 3; 91.4% of initial at day 7).

Concentration analysis: The concentration ranges for the dose preparations were all >90% of nominal.

The analytical data indicated that the concentration and stability of terbufos in the corn oil preparations were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Twice daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals. Gross observations of the dams were conducted daily.

Ten dams per group were observed outside the home cage at least twice during the gestation dosing period (days 7 and 14) and twice during the lactation dosing period (days 7 and 14). The following functional observations were recorded.

	Functional observations–Maternal animals
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalmus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis. 6) Respiration 7) Activity/arousal level
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded upon arrival at the testing facility (GD 0) and on GDs 6-20. Females with litters were weighed on the day of parturition and on LDs 10, 14 and 21. Food consumption measurements were recorded on GDs 0, 6, 13 and 20 and on LDs 1, 7, 14 and 21.

Blood samples were collected from the retroorbital venous flexus under isoflurane anesthesia from 10 dams per group on PND 21 for serum and red blood cell cholinesterase measurements. Brain cholinesterase was measured on the same animals on PND 21.

b. Offspring:

1. **Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day) 0. Live pups were counted, sexed and weighed individually for each litter on postnatal days 1, 4 (before standardization) and 11, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity.

   On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible), with the exception of the high dose group; excess pups were killed and discarded. Litters with fewer than 8 pups were removed from the study.

2. **Developmental landmarks:** Beginning on postnatal day 40, male offspring were examined daily for preputial separation. Beginning on postnatal day 27, female offspring were examined daily for vaginal patency. The age of onset and the offspring body weight at that time were recorded.

3. **Postweaning observations:** After weaning on postnatal day 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly.

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

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

   ii) **Motor activity testing (subset IV):** Motor activity was evaluated in 10 rats/sex/dose on days 13, 17, 21 and 60 using the Tru Scan Photobeam Linc. The activity was measured in 10 enclosures in randomized order. Each enclosure was equipped with two sensor rings each with 16 light beams per cage side. The distance covered and the number of rearings were recorded over 12 intervals, each lasting 5 minutes. No food or water was provided and the room was darkened during the measurements.

   iii) **Auditory startle reflex habituation (subset III):** Auditory startle reflex habituation testing was performed on 10 offspring/sex/dose on postnatal days 24 and 60, using the SR-LAB; STARTLE RESPONSE SYSTEM. The animals were allowed a 5 minute acclimation period in the response chamber with a 70dBA background noise. The startle response was recorded in 50 trials with a startle stimulus sound level of 120 dBA with a 5 second interval between the trials. Response was recorded for 50 milliseconds.

Maximum amplitude and latency to peak response were analyzed in 5 blocks of 10 trials each.

iv) **Learning and memory testing (subsets V, VI):** Learning and memory testing was performed in 10 offspring/sex/dose. Water maze testing was performed on postnatal days 23 and 30 (subset V) and on postnatal days 60 and 67 (subset VI). The testing consisted of three parts and was performed in two weeks, beginning with learning ability (learning 1) in the first week, followed by memory and relearning ability (learning 2) in the second week. The learning 1 test consisted of 6 trials at intervals of 1 hour for each animal. At each trial, the animals were required to find an escape ladder on the right side of an M-shaped water maze pool. The maximum duration of swimming was 6 minutes per trial. A positive score (+) was given if the animal found the escape immediately. If the animal went in the wrong direction, it was given a negative (-) score but left in the water until it found the ladder or the 6 minutes expired. The time needed to find the ladder was also recorded. For the memory test, the same animals had to find the ladder on the right side of the pool after one week; the time needed to find the ladder was recorded. The learning 2 test started 1 hour after completion of the memory test. The same procedures were followed as in learning test 1, except the ladder was placed on the left side of the maze. The initial trials in the learning 1 and 2 tests were not included in the analyses since they served as an acclimation to the test.

5) **Cholinesterase determination:** Cholinesterase (serum, red blood cell and brain) activity was determined on PNDs 4 (using culled pups) and 21 (3 hours after dosing, 1 pup/sex/litter). Blood samples were collected from the vena cava cranialis after decapitation following isoflurane anesthesia. The same animals were used for blood and brain analyses. The blood and brain samples were analyzed using a spectrophotometric procedure, based on modified Ellman's methods that were adapted to a Cobas Fara analyzer. Red blood cell samples were measured using DTNA as chromogen. Blood and brain samples were kept on ice during collection and processed as soon as possible. After all the brains were collected, the samples were deep frozen and stored at -80^{0}C until analysis. Hematocrit or protein content of the brain were determined in order to calculate the cholinesterase activity of red blood cells per liter erythrocytes and specific cholinesterase activity of the brain.

5. **Postmortem observations**:

a. **Maternal animals:** The dams were sacrificed by cervical dislocation and discarded without examination on PND 21 after the pups were weaned. Animals without a litter were discarded after the uterus had been stained for evidence of early resorptions. To determine the number of implantation sites, the uterus was stained with 10% ammonium sulfide solution.

b. **Offspring:** All pups selected for a subset which died were examined externally, eviscerated and their organs assessed macroscopically and fixed in 4% formaldehyde. All pups with scheduled sacrifice (pups sacrificed on PND 21 and subset IV, V and VI) were killed by cervical dislocation and discarded without examination.

On PND 11, 10 animals/sex/group and study section were subjected to deep anesthesia and sacrificed by exsanguination. The skull was separated from the body and stored in neutrally buffered 4% formaldehyde.

At postnatal days 22 and 60 (±2), ten pups/sex/group and study section were selected for brain weight measurements and neuropathology. The animals were first weighed and then deeply anesthetized and sacrificed by perfusion fixation. SOERENSEN phosphate buffer was used as a rinsing solution and neutrally buffered 4% formaldehyde as a fixative. The animals were necropsied and the visible organs assessed by gross pathology. The cranial vault and spinal cord were opened and the skin removed from both hind extremities. Brain (with olfactory bulb) weight was determined after removal of the organ. The perfused animals were stored in a neutrally buffered 4% formaldehyde solution for at least 48 hours. The length and maximum width of the cerebrum and cerebellum were measured.

On PND 22, the following organs/tissues from pups fixed by perfusion were processed histotechnically:

Brain with olfactory bulb
Pituitary gland
Eyes with retina and optical nerve
Gasserian ganglia with nerve
Spinal cord - cervical cord (C1-C5), thoracic cord (T5-T8), lumbar (L1-L4)
Gastrocnemius muscle
Nose, nasal cavity

On PND 60 (±2), the same tissues from PND 22 were processed, in addition to the following:

Dorsal root ganglion (C1-C5[3x] and L1-L4[3x])
Dorsal root fiber (C3-C6 and L1-L4)
Ventral root fiber (C3-C6 and L1-L4)
Proximal sciatic nerve
Proximal tibial nerve (at knee)
Distal tibial nerve (at lower leg)

The following organs were processed on PNDs 22 and 60 (±2) as described.

Organ samples from:	Dose groups (mg/kg/day)			
	0	0.01	0.08	0.15
Brain (cross sections):				
- Olfactory bulb	A10	B10	B10	A10
- Frontal lobe	A10	C10	C10	A10
- Parietal lobe with diencephalon	A10	B10	B10	A10
- Midbrain with occipital and temporal lobe	A10	B10	B10	A10
- Pons	A10	B10	B10	A10
- Cerebellum (2 planes of section)	A10	B10	B10	A10
- Medulla oblongata	A10	B10	B10	A10
Spinal cord (longitudinal and cross sections)				
- Cervical cord I (C1-C3: **C1**)	A10	F10	F10	A10
- Cervical cord II (C3-C5: **C5**)	A10	F10	F10	A10
- Thoracic cord (T5-T8: **T8**)	A10	F10	F10	A10
- Lumbar cord (L1-L4: **L4**)	A10	F10	F10	A10
Brain-associated organs/tissues				
- Eyes with retina and optical nerve	A10	F10	F10	A10
- Pituitary gland	A10	F10	F10	A10
- Olfactory epithelium (nose cavity, level III)	A10	F10	F10	A10
Peripheral nervous system:				
- Gasserian ganglia with nerve	A10	F10	F10	A10
- Gastrocnemius muscle (longitudinal and cross-sections)	A10	F10	F10	A10

A= Paraplast embedding, sectioning and staining with hematoxylin-eosin (HE).
B=Paraplast embedding;
C=Sectioning and staining with hematoxylin-eosin - male animals of PND 62 subset only;
F=Preservation in neutrally buffered, 4% formaldehyde solution;
10=All perfused animals per group per sex. The HE stained sections were examined by light microscopy and assessed.

Morphometric measurements of major brain areas were done in the same animals selected for neuropathology.

The brain areas measured for thickness were:

Neocortex (frontal and parietal cortices)

Caudate nucleus/putamen- the largest lateral extension of the left and right part

Hippocampus- the largest dorsoventral extension

Corpus callosum- the width was measured at the middle line of the cross section

Cerebellum- the width of a select folium was measured in the middle of a line which runs vertically to a tangent from the tip to the base of the folium.

D. DATA ANALYSIS:

1. **Statistical analyses:** The following parameters were analyzed using the Dunnett's test (two-sided) for the hypothesis of equal means: food consumption (females), body weight and body weight gain (females and pups), duration of gestation and number of pups delivered per litter.

   The following were analyzed using the Fisher's Exact test for hypothesis of equal proportions: female fertility index, gestation index, females with live born pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, and water maze evaluation.

   The Wilcoxon test (one-sided) for the hypothesis of equal medians was also used for water maze evaluation.

   Motor activity and startle response were analyzed using Kruskal-Wallis test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the control was performed using Mann-Whitney U-test (two-sided) for the hypothesis of equal medians.

   Cholinesterase and brain weight (absolute and relative) data were analyzed using Kruskal-Wallis test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the control was performed using Wilcoxon test (two-sided) for the hypothesis of equal medians.

   Morphometric parameters were analyzed using the Wilcoxon-test (one-sided) with Bonferoni-Holm-Adjustment for the hypothesis of equal medians.

2. **Indices:**

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

   $$\text{Female fertility index (\%)} = \frac{\text{number of pregnant females*}}{\text{number of females mated**}} \times 100$$

   * defined as number of females that gave birth to a litter or with pups/fetuses *in utero*
   ** defined as number of females with vaginal sperm or that gave birth to a litter or with fetuses *in utero*

Gestation index (%) = number of females with live pups on the day of birth x 100
number of females pregnant*

* defined as the number of females that gave birth to a litter or with fetuses *in utero*

Live birth index (%) = number of live born pups at birth x 100
number of pups born

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

Viability index (%) = number of live pups on day 4* after birth x 100
number of live pups on day of birth

* before standardization of litters

Lactation index (%) = number of live pups on day 21 after birth x100
number of live pups on day 4* after birth

* after standardization of litters

Sex ratio = number of live male or female pups on day 0/21 x 100
number of live male or female pups on day 0/21

3. **Positive and historical control data:** No positive control data were provided. Historical control data from five studies using Wistar rats were provided for the following parameters: motor activity (distance and rearing on PNDs 13, 17, 21 and 60) and auditory startle response (maximum amplitude and latency to peak on PNDs 24 and 60).

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** There were no treatment-related maternal deaths during gestation or lactation. One high-dose dam died on lactation day 7 because of gavage error. There were no treatment-related clinical signs observed during gestation or lactation.

2. **Body weight and food consumption**: Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 2. No treatment-related effects on body weight were noted in maternal animals during gestation or lactation. Decreased mean body weight was observed in high-dose dams on lactation day 5 (6% decrease, $p \leq 0.05$) and lactation day 8 (6% decrease, $p \leq 0.05$) only. In the absence of a sustained decrease in body weight, these observations are considered incidental to treatment.

   Food consumption in treated animals was comparable to controls during gestation and lactation.

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

Observations/study interval	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Gestation (n= 25-30)				
Body wt. Gestation day 0 (g)	158.0±11.25	157.1±11.01	160.7±11.41	158.2±10.83
Body wt. Gestation day 7 (g)	191.3±13.42	189.4±13.04	190.2±12.90	190.4±10.27
Body wt. Gestation day 15 (g)	229.4±17.54	225.5±15.14	227.8±17.16	224.0±12.43
Body wt. Gestation day 20(g)	274.0±22.00	270.2±19.48	274.4±21.67	266.8±16.63
Wt. gain gestation days 0-20 (g)	116.0±16.56	113.1±14.18	113.7±14.78	108.6±14.37
Food consumption gestation days 0-6 (g/animal/day)	16.9±1.75	17.0±1.46	16.3±1.41	16.7±1.14
Food consumption gestation days 6-13 (g/animal/day)	18.2±2.23	17.8±1.77	17.7±2.19	17.6±1.41
Food consumption gestation days 13-20 (g/animal/day	19.0±2.12	18.5±1.86	18.9±2.43	18.7±1.75
Lactation (n=25-42)				
Body wt. lactation day 0 (g)	214.6±18.71	215.0±16.38	217.9±22.16	211.1±15.37
Body wt. lactation day 5 (g)	225.2±16.59	220.0±15.66	220.5±18.26	212.3*±12.82 (5.7)
Body wt. lactation day 8 (g)	235.8±18.33	229.8±15.67	231.0±17.63	222.7*±12.88 (5.6)
Body wt. lactation day 14 (g)	247.1±20.15	240.3±16.99	242.9±18.63	237.7±12.07
Body wt. lactation day 21 (g)	239.8±17.30	236.1±15.85	237.6±17.19	235.8±10.63
Wt gain lactation days 0-21(g)	23.1±10.43	22.0±11.43	21.5±9.17	24.2±7.91
Food consumption lactation days 1-7 (g/animal/day)	29.1±2.47	28.6±2.87	29.0±2.97	28.0±2.45
Food consumption lactation days 7-14 (g/animal/day)	41.8±2.54	41.5±2.20	42.0±3.06	40.7±2.74
Food consumption lactation days 14-21 (g/animal/day)	50.0±3.36	49.4±2.45	50.4±3.90	49.1±3.44

[a]Data obtained from pages 98-107. MRID 46214301

* Statistically significantly different from control, $p \leq 0.05$

Number in parentheses is % decrease compared to control, calculated by reviewer.

3. **Reproductive performance**: There were no treatment-related effects on fertility, gestation indices or gestation length. Results for the maternal animals are summarized in Table 3.

TABLE 3. Reproductive performance [a]

Observation	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Number mated	41	42	42	42
Number pregnant	38	35	42	40
Fertility index (%)	93	83	100	95
Gestation index (%)	100	100	100	98
Mean (±SD) gestation duration (days)	21.9±0.39	21.9±0.55	21.9±0.45	21.8±0.51

[a] Data obtained from page108 in the study report. MRID 46214301
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

4. **Cholinesterase activity**: High-dose dams exhibited toxicologically significant decreases in erythrocyte (26% inhibition; $p \leq 0.01$) and brain (33% inhibition; $p \leq 0.01$) cholinesterase activities on PND 21. Erythrocyte cholinesterase activity was inhibited by 13% ($p \leq 0.05$) in mid-dose dams; however, no effect was seen in brain cholinesterase and the toxicological significance was not certain due to this isolated small magnitude of change. No other cholinesterase activity effects were noted in dams on PND 21. The data are included with offspring data in Table 14.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 4. There were no treatment-related effects on the average number of delivered pups per dam. However, the number of liveborn pups was lower in the high-dose group, and the number of stillborn pups was also increased relative to the controls. This is due primarily to the fact that the entire litter (9 pups) of one high-dose dam was dead by day 1. Eight of the pups were stillborn, and the ninth died on day 1. This is considered incidental.

   The sex ratio of live pups on the day of birth was not affected by treatment. There were no treatment-related clinical signs of toxicity.

TABLE 4. Litter size and viability [a]				
Observation	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Total number born	335	305	382	336
Pups/dam delivered	8.8±1.57	8.7±1.54	9.1±1.82	8.6±1.55
Number of litters born	38	35	42	39
Number with stillborn pups	4	2	3	8
Number born live	331	299	378	321**
Number born dead	4	6	4	15**
Sex Ratio Day 0 (% ♂)	52.3	48.5	45.5	50.2
Mean litter size.				
Day 0	8.7±1.52	8.5±1.99	9.0±1.95	8.2±1.98
Day 4 [b]	7.8±3.05	7.3±3.63	8.0±3.40	7.6±3.03
Day 4 [c]	5.9±3.57	5.7±3.67	5.7±3.66	5.7±3.65
Day 11	5.9±3.57	5.7±3.67	5.7±3.66	5.3±3.82
Day 17	5.3±3.26	5.1±3.27	5.2±3.37	4.7±3.43
Day 21	5.3±3.24	5.0±3.24	5.2±3.34	4.7±3.40
Live birth index	99	98	99	96

[a] Data obtained from pages 108-111 in the study report. MRID 46214301
[b] Before standardization (culling).
[c] After standardization (culling).
** Statistically different from control. $p<0.01$

2. **Body weight:** Male and female offspring body weight was approximately 4-6% ($p<0.05$) lower for high-dose animals from LD 11 through weaning at day 21 (Table 5). Body weight gain was decreased for high-dose males and females during lactation days 1-11 (up to 11% decrease; $p<0.05$) and lactation days 12-13 (up to 14% decrease; $p<0.01$). No body weight effects were noted for low- or mid-dose pups during lactation.

TABLE 5. Selected mean (±SD) pre-weaning pup body weights and body weight gain (g) [a]								
PND	Dose (mg/kg/day)							
	0	0.01	0.08	0.15	0	0.01	0.08	0.15
	Males				Females			
Body weight (g)								
1	6.6±0.48	6.6±0.52	6.7±0.69	6.5±0.45	6.3±0.46	6.3±0.49	6.4±0.61	6.2±0.56
4[b]	10.3±0.74	10.3±0.83	10.3±1.12	10.0±0.90	10.1±0.76	10.0±0.82	10.0±1.04	9.7±0.96
4[c]	10.3±0.74	10.3±0.84	10.3±1.13	10.0±0.90	10.1±0.73	10.0±0.83	10.0±1.05	9.7±0.98
11	23.5±1.53	23.6±1.65	23.7±2.02	22.3±1.97 (5.1)	23.1±1.65	23.1±1.58	23.2±1.88	21.8*±2.10 (5.6)
16	35.0±2.11	35.4±2.25	35.2±2.68	33.3*±2.87 (4.9)	34.3±1.95	34.4±1.99	34.4±2.40	32.6*±2.60 (5.0)
21	48.6±3.44	48.9±3.29	48.5±3.88	46.7±4.01 (3.9)	47.4±3.01	47.7±2.55	47.4±3.45	45.4±3.69 (4.2)
Body weight gain (g)								
1-4	3.7±0.37	3.7±0.41	3.6±0.56	3.5±0.54 (5.4)	3.8±0.40	3.7±0.44	3.6±0.55	3.4*±0.52 (11)
4-11	13.2±1.12	13.3±1.28	13.4±1.35	12.3±1.38 (6.8)	13.0±1.23	13.1±1.22	13.2±1.25	12.1*±1.42 (6.9)
12-13	2.2±0.41	2.2±0.28	2.0±0.36	2.0±0.28 (9.1)	2.1±0.34	2.2±0.28	2.0±0.32	1.8**±0.21 (14)
4-21	38.3±3.10	38.6±2.91	38.2±3.44	36.6±3.38 (4.4)	37.3±2.79	37.7±2.25	37.4±3.03	35.7±3.01 (4.3)

PND = post-natal day
N=25-30
[a] Data obtained from pages 112-121. MRID 46214301.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically significantly different from control, $p \leq 0.05$
** Statistically significantly different from control, $p \leq 0.01$.
Number in parentheses is % decrease from control value, calculated by reviewer

Body weight was measured in male and female pups in subsets III and IV (weeks 0-5 post-weaning), V (weeks 0-1 post-weaning) and VI (weeks 0-6 post-weaning). In subset V, body weight of high-dose males ($p \leq 0.05$) and females (N.S.) was 9% lower than controls during week 0 after weaning. However, these animals gained approximately 9% more than controls during week 0-1, and any body weight difference had resolved by week 1-2. Body weight gain of subset III low-dose males was increased 20% ($p \leq 0.05$) during week 4-5, and body weight gain of subset V low-dose males was increased 9% during week 1-2. None of the post-weaning body weight observations were considered toxicologically-significant. Selected subset VI (chosen for tabulation because observation period was longest of any subset) mean post-weaning offspring body weight data are presented in Table 6.

TABLE 6. Selected mean (±SD) post-weaning subset VI pup body weights and body weight gain (g) [a]								
Post-weaning week	Dose (mg/kg/day)							
	0	0.01	0.08	0.15	0	0.01	0.08	0.15
	Males				Females			
Body weight (g)								
0	53.2±6.71	51.8±4.79	52.8±5.74	49.7±4.73	52.2±5.45	54.6±4.64	50.3±5.89	47.8±2.35
1	93.7±8.58	92.3±7.04	91.6±11.38	89.5±6.41	87.7±6.59	91.8±6.69	86.2±8.87	84.6±4.18
2	140.3±10.03	138.5±9.24	137.7±16.11	133.9±7.45	121.9±7.59	125.7±7.07	116.7±8.55	119.2±5.26
3	183.3±10.92	180.5±13.94	178.5±23.48	175.1±9.62	141.9±7.86	147.1±8.29	138.7±9.81	141.5±8.17
4	224.6±13.81	221.0±16.88	212.8±38.38	212.9±10.92	155.6±9.57	163.8±7.37	153.5±15.72	157.4±6.50
5	262.7±16.05	259.0±18.47	251.3±35.47	247.4±11.33	170.2±11.09	176.4±8.51	167.2±18.26	171.6±7.35
6	291.121.29	288.5±21.24	279.2±36.33	276.3±15.25	180.2±12.64	187.8±12.04	176.1±13.11	183.2±9.06
Body weight Gain (g)								
0-6	237.8±19.75	236.7±20.76	226.4±33.36	226.6±13.41	128.1±12.61	133.2±9.25.	125.8±10.88	135.4±8.14

[a] Data obtained from pages 145-148. MRID 46214301.

3. Developmental landmarks:

Sexual maturation: No treatment-related effects on preputial separation in males or vaginal opening in females were noted. Data are summarized in Table 7.

Table 7. Mean (±SD) age of sexual maturation (days)[a]				
Parameter	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
N (M/F)	30/30	30/30	29/30	30/30
Preputial separation (males)	44.2±2.14	42.8**±1.48	43.2±1.41	44.1±1.87
Vaginal opening (females)	31.5±2.27	31.8±2.37	31.5±2.06	30.8±1.74

[a] Data obtained from pages 123-124. MRID 46214301
**$p \leq 0.01$.

4. Behavioral assessments:

a. **Functional observational battery (subset IV)**: There were no treatment-related FOB findings in offspring at any dose level on any test day (PNDs 4, 11, 21, 35, 45 or 60).

b. **Motor/locomotor activity (subset IV):** Total movement activity and rearing of offspring are shown in Tables 8 and 9, respectively. On PND 13, motor activity was increased 35% and 58% in males and 34% and 19% in females at the mid- and high-dose groups, respectively. On PND 17, motor activity was increased 18% and 26% in males and 15% and 64% in females at the mid-and high-dose groups, respectively (Table 8). These increases were attributed to treatment. Motor activity in males and females at the low dose on PND 21 and 60 were unaffected by treatment. Rearing (Table 9) was unaffected by treatment.

TABLE 8. Total (±S.D.) motor activity data (total distance traveled (cm) for session) [a]

Test Day	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Males				
PND 13	2011.9±834.1	2281.1±1213.2 (17)	2706.9±1195.5(35)	3182.6±1519.3 (58)
PND 17	3167.6±1910.1	3731.2±2415.2 (18)	3742.9±1754.1 (18)	3977.9±1405.3 (26)
PND 21	3551.6±1119.7	3477.1±848.8	3922.9±1501.0	4085.6±1248.5
PND 60	7391.8±1337.7	7569.3±2698.8	8452.4±1139.1	7315.3±1653.3
Females				
PND 13	2226.2±991.4	2897.1±2046.3	2990.6±1459.2 (34)	2649.1±1160.0 (19)
PND 17	3196.5±1631.1	3002.0±1858.9	3660.3±1307.7 (15)	5257.4**±1622.6 (64)
PND 21	3683.8±1157.3	3081.7±1229.0	4131.0±1337.8	4241.1±1149.3
PND 60	10055±2678.8	9156.2±2009.1	9374.6±1848.4	10180±1939.5

[a] Data obtained from pages 217-232. MRID 46214301
N =9-10
** Statistically different from control. $p<0.01$
Number in parantheses is % increase compared to control value calculated by reviewer

TABLE 9. Total (±S.D.) rearing data for session [a]

Test Day	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Males				
PND 13	75.7±29.4	103.7±52.8	121.9±78.9	83.1±73.7
PND 17	127.9±84.1	138.3±75.5	146.2±63.6	102.2±57.7
PND 21	140.8±47.9	124.2±37.6	136.8±64.0	136.9±45.9
PND 60	181.0±34.6	176.2±59.0	222.7±59.5	182.2±42.3
Females				
PND 13	61.7±28.2	95.9±62.9	108.1±59.6	55.8±37.5
PND 17	105.0±45.1	99.9±52.3	131.3±45.8	150.9±51.4
PND 21	136.3±44.1	109.8±40.4	153.2±56.6	140.8±44.8
PND 60	267.6±73.5	222.2±55.7	255.9±56.0	231.2±38.2

[a] Data obtained from pages 233-248. MRID 46214301
N =9-10

c. **Auditory startle reflex (subset III) :** The amplitude and habituation data are presented in Tables 10 and 11. There were no treatment-related effects on startle amplitude, latency, or habituation in either sex at any dose-level on any test day. A decrease in latency ($p \leq 0.05$) was observed in high- and mid-dose males on day 24 (block 4). This isolated finding was not considered to be treatment-related.

TABLE 10. Auditory startle amplitude (v) (mean ±SD) [a]

	Trial Block	Dose (mg/kg/day)			
		0	0.01	0.08	0.15
Males					
PND 24	1	399.3±131.1	399.2±88.7	529.7±194.7	373.0±133.0
	2	417.6±152.0	389.5±136.5	491.4±277.2	326.0±112.1
	3	338.7±111.0	380.3±133.9	468.5±194.9	324.3±104.0
	4	350.4±147.6	352.9±111.6	474.9±161.8	328.2±111.6
	5	389.5±137.0	354.4±64.8	453.2±135.5	306.8±37.4
	Mean	379.1±109.2	375.2±81.0	483.6±160.8	331.6±80.5
PND 60	1	1049.0±587.2	794.1±458.6	1222.0±651.8	865.2±254.2
	2	876.6±711.3	538.3±234.7	887.3±530.3	538.5±282.0
	3	878.5±1079.7	476.6±192.4	733.6±640.0	463.2±305.8
	4	813.7±828.9	423.0±186.5	690.5±649.2	434.7±289.1
	5	749.0±800.7	461.1±220.0	639.7±378.1	443.8±248.5
	Mean	873.4±734.7	538.6±215.5	834.6±532.9	549.1±254.5
Females					
PND 24	1	416.6±93.4	380.1±112.3	329.9±73.5	368.9±89.7
	2	350.1±120.9	368.8±103.5	316.6±89.2	334.4±74.5
	3	350.5±140.2	300.2±90.8	347.4±76.3	371.7±110.3
	4	339.2±124.0	328.0±122.5	344.3±109.3	372.9±101.1
	5	392.5±136.3	314.1±103.2	319.9±70.4	430.6±154.9
	Mean	369.8±113.2	338.3±91.3	331.6±51.2	375.7±89.3
PND 60	1	814.4±487.5	889.9±553.6	586.5±272.3	861.3±476.6
	2	577.1±300.6	863.0±590.9	464.3±213.4	701.5±511.5
	3	525.8±310.0	579.6±472.9	374.9±135.2	649.4±552.1
	4	534.5±294.7	576.6±467.0	327.5±142.1	560.8±391.8
	5	451.0±286.3	445.2±294.0	365.6±185.7	453.7±325.2
	Mean	580.6±309.3	670.9±421.8	423.8±170.3	645.3±381.7

[a]Data obtained from 249-252, MRID 46214301.
N=10

TABLE 11. Auditory startle: time to peak amplitude (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	0.01	0.08	0.15
Males					
PND 24	1	43.3±16.3	34.2±8.9	33.0±5.7	35.4±14.6
	2	35.4±7.6	28.9±6.5	31.7±5.2	28.8±7.0
	3	32.9±7.5	29.1±7.5	28.8±5.4	26.4±7.2
	4	33.5±6.7	30.4±5.3	25.5*±4.6	24.4**±1.7
	5	31.2±5.9	28.0±6.0	26.9±4.9	28.6±6.0
	Mean	35.2±6.9	30.1±4.5	29.2±3.5	28.7±5.2
PND 60	1	45.1±16.0	35.8±10.9	42.0±13.4	42.5±6.0
	2	34.1±9.7	27.8±3.4	32.6±8.0	32.3±6.9
	3	32.4±11.2	27.0±3.6	30.0±7.1	28.3±7.3
	4	32.8±12.8	26.3±5.8	27.4±7.9	28.6±5.8
	5	31.2±11.4	25.2±3.6	30.0±5.8	29.3±5.7
	Mean	35.1±11.0	28.4±3.2	32.4±7.1	32.2±4.2
Females					
PND 24	1	35.2±10.5	32.1±5.2	34.3±7.5	31.0±7.6
	2	29.9±7.6	29.0±5.2	31.7±4.9	28.6±5.4
	3	31.4±6.9	27.2±4.2	28.0±3.5	28.6±5.9
	4	26.6±6.8	29.4±8.0	27.3±2.7	28.3±4.8
	5	26.7±6.0	30.2±5.7	25.2±3.2	26.3±8.0
	Mean	30.0±5.1	29.6±3.8	29.3±2.7	28.5±4.1
PND 60	1	40.4±15.6	42.0±16.0	36.1±8.9	36.2±9.3
	2	33.2±7.9	36.0±13.3	32.5±9.4	34.8±13.4
	3	32.5±11.5	31.8±11.5	28.5±5.9	35.0±12.0
	4	30.9±9.5	34.0±16.5	26.8±4.9	31.3±8.1
	5	26.7±6.5	28.3±7.4	25.5±3.8	29.9±5.5
	Mean	32.7±8.8	34.4±10.2	29.9±3.7	33.5±8.2

[a]Data obtained from pages 253-256. MRID 46214301
N=10
* Statistically different from control. $p<0.05$
** Statistically different from control. $p<0.01$

d. <u>Learning and memory testing (subsets V and VI)</u>: There were no treatment-related effects in either sex in any dose group. In subset VI (PND 60), a decreased ($p \leq 0.05$) number of successful mid-dose females was noted in trial 3. This isolated finding is not considered to be treatment-related. Data are summarized in Tables 12 and 13.

TABLE 12. Water maze performance in PND 23 offspring [a]					
Session/parameter		Dose (mg/kg/day)			
		0	0.01	0.08	0.15
Males					
Number (%) Animals Reaching Criteria					
Learning 1	Trial 2	4 (40)	4 (40)	7 (70)	8 (80)
	Trial 3	7 (70)	3 (30)	8 (80)	6 (60)
	Trial 4	6 (60)	8 (80)	8 (80)	9 (90)
	Trial 5	7 (70)	9 (90)	7 (70)	8 (80)
	Trial 6	8 (80)	8 (80)	8 (80)	10 (100)
Memory		8 (80)	8 (80)	8 (80)	10 (100)
Learning 2	Trial 2	0 (0)	2 (20)	3 (30)	0 (0)
	Trial 3	1 (10)	3 (30)	4 (40)	2 (20)
	Trial 4	4 (40)	3 (30)	5 (50)	4 (40)
	Trial 5	2 (20)	5 (50)	6 (60)	6 (60)
	Trial 6	4 (40)	5 (50)	4 (40)	3 (30)
Females					
Number (%) Animals Reaching Criteria					
Learning 1	Trial 2	6 (60)	6 (60)	4 (40)	3 (30)
	Trial 3	7 (70)	6 (60)	8 (80)	6 (60)
	Trial 4	7 (70)	8 (80)	9 (90)	6 (60)
	Trial 5	8 (80)	10 (100)	9 (90)	9 (90)
	Trial 6	8 (80)	9 (90)	9 (90)	8 (80)
Memory		9 (90)	9 (90)	9 (90)	9 (90)
Learning 2	Trial 2	2 (20)	2 (20)	2 (20)	1 (10)
	Trial 3	2 (20)	4 (40)	4 (40)	3 (30)
	Trial 4	4 (40)	6 (60)	4 (40)	3 (30)
	Trial 5	4 (40)	7 (70)	2 (20)	5 (50)
	Trial 6	4 (40)	7 (70)	3 (30)	6 (60)

[a] Data obtained from pages 149-150. MRID 46214301.
N=10

TABLE 13. Water maze performance in PND 60 offspring [a]

Session/parameter		Dose (mg/kg/day)			
		0	0.01	0.08	0.15
Males					
Number (%) Animals Reaching Criteria					
Learning 1	Trial 2	4 (40)	6 (60)	3 (30)	6 (60)
	Trial 3	8 (80)	7 (70)	6 (60)	5 (50)
	Trial 4	8 (80)	6 (60)	5 (50)	9 (90)
	Trial 5	8 (80)	7 (70)	8 (80)	9 (90)
	Trial 6	9 (90)	9 (90)	9 (90)	8 (80)
Memory		9 (90)	9 (90)	9 (90)	9 (90)
Learning 2	Trial 2	3 (30)	3 (30)	2 (20)	1 (10)
	Trial 3	5 (50)	4 (40)	7 (70)	4 (40)
	Trial 4	5 (50)	7 (70)	7 (70)	6 (60)
	Trial 5	7 (70)	8 (80)	8 (80)	8 (80)
	Trial 6	8 (80)	10 (100)	8 (80)	8 (80)
Females					
Number (%) Animals Reaching Criteria					
Learning 1	Trial 2	5 (50)	5 (50)	5 (50)	5 (50)
	Trial 3	7 (70)	5 (50)	6 (60)	6 (60)
	Trial 4	7 (70)	5 (50)	5 (50)	7 (70)
	Trial 5	5 (50)	6 (60)	4 (40)	7 (70)
	Trial 6	8 (80)	7 (70)	6 (60)	8 (80)
Memory		8 (80)	6 (60)	7 (70)	8 (80)
Learning 2	Trial 2	4 (40)	1 (10)	1 (10)	3 (30)
	Trial 3	7 (70)	4 (40)	2* (20)	6 (60)
	Trial 4	6 (60)	5 (50)	4 (40)	6 (60)
	Trial 5	6 (60)	5 (50)	6 (60)	5 (50)
	Trial 6	6 (60)	5 (50)	6 (60)	6 (60)

[a] Data obtained from pages 151-152 in the study report. MRID 46214301.
* Statistically different from control. $p<0.05$
N=10

5. **Cholinesterase activity:** Results of cholinesterase (ChE) activity assessment are presented in Table14. No treatment-related changes were noted in serum, erythrocyte or plasma cholinesterase activity for male or female pups on PND 4.

   On PND 21, significant dose-related inhibition ($p \leq 0.05$ or 0.01) in serum (56-86% inhibition), erythrocyte (47-84% inhibition), and brain (38-75% inhibition) ChE activity were observed in mid- and high-dose males and females. No treatment-related effects were noted for low-dose animals.

TABLE 14. Cholinesterase activity in dams and offspring				
Cholinesterase [mean ± SD (% inhibition relative to control)]	**Dose (mg/kg/day)**			
	0	**0.01**	**0.08**	**0.15**
Lactation day 21 dams				
Serum (µkat/L)	17.07 ±3.14	18.11± 4.03	16.83±3.35	18.75±3.37
RBC (µkat/L)	33.09±4.82	32.74±4.67	28 77*±2.96 (-13)	**24.50**±4.57 (-26)**
Brain (µkat/g)	2.88 ± 0.70	2.73±1.26	2.65±0.92 (8)	**1.93**±0.44 (-33)**
Day 4 male offspring				
Serum (µkat/L)	13.58 ±1.34	13.53± 1.17	13.60±0.87	13.74±1.09
RBC (µkat/L)	15.08±3.21	11.28±3.67	15.24±3.70	12.86±3.92
Brain (µkat/g)	1.18± 0.05	1.21±0.11	1.17±0.06	1.14±0.08
Day 4 female offspring				
Serum (µkat/L)	13.72 ±1.00	13.30± 1.06	13.76±1.59	13.78±1.12
RBC (µkat/L)	13.62±3.78	15.63±4.60	16.06±3.73	14.18±2.83
Brain (µkat/g)	1.14± 0.06	1.18±0.10	1.17±0.08	1.16±0.07
Day 21 male offspring				
Serum (µkat/L)	13.68±2.30	13.33± 1.70	**5.42**±1.04 (-60)**	**1.86**±0.38 (-86)**
RBC (µkat/L)	35.10±8.33	39.18±8.21	**18.51**±3.91 (-477)**	**5.51**±1.69 (-84)**
Brain (µkat/g)	2.32± 0.33	2.26±0.49	**1.36**±0.20 (-41.)**	**0.57**±0.17 (-75)**
Day 21 female offspring				
Serum (µkat/L)	13.47±2.13	13.74± 1.89	**5.92**±1.16 (-56)**	**2.10**±0.50 (-84)**
RBC (µkat/L)	36.48±9.79	35.77±7.47	**17.45**±2.36 (-52)**	**5.86**±0.91 (-83)**
Brain (µkat/g)	2.30± 0.41	2.41±0.25	**1.42**±0.37 (-38)**	**0.59**±0.10 (-74)**

Data obtained from pages 281-285. MRID 46214301.
N=10
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

6. Postmortem results (subsets II and III):

Brain weights: No treatment-related effects were noted on PND 22 or PND 60 (±2). Mean brain weight data are presented in Table 15.

TABLE 15. Mean (±SD) Brain Weight Data in Offspring [a]				
Parameter	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Males				
Day 22				
Terminal body weight (g)	52.03±3.775	52.07±4.336	49.13±4.49	48.67±3.384
Brain weight (g)	1.641±0.063	1.632±0.057	1.62±0.082	1.627±0.067
Brain-to-body weight ratio (%)	3.165±0.191	3.152±0.263	3.32±0.328	3.352±0.174
Day 60 (±2)				
Terminal body weight (g)	267.41±18.89	285.84±21.44	275.05±27.05	260.91±25.94
Brain weight (g)	2.006±0.058	2.072±0.102	2.003±0.092	1.989±0.072
Brain-to-body weight ratio (%)	0.753±0.05	0.727±0.047	0.732±0.046	0.767±0.057
Females				
Day 22				
Terminal body weight (g)	49.14±3.94	50.85±2.325	48.41±4.717	47.49±3.276
Brain weight (g)	1.56±0.038	1.601±0.043	1.581±0.046	1.596±0.057
Brain-to-body weight ratio (%)	3.19±0.227	3.153±0.136	3.261±0.258	3.371±0.191
Day 60 (±2)				
Terminal body weight (g)	181.19±15.19	177.17±14.48	182.96±15.42	183.91±13.29
Brain weight (g)	1.896±0.073	1.863±0.04	1.914±0.068	1.906±0.073
Brain-to-body weight ratio (%)	1.052±0.079	1.058±0.089	1.052±0.082	1.039±0.053

[a] Data obtained from pages 286-293, MRID 46214301
N=10

C. Neuropathology (subsets II and III)

1. **Macroscopic examination:** No treatment-related effects were reported for male or female offspring on PND 22 or 60 (±2).

2. **Microscopic examination:** No treatment-related effects were reported for male or female offspring on PNDs 22 and 60 (±2).

3. **Brain Morphometry:** Morphometric evaluation, presented in Table 16, revealed a statistically significant decrease ($p \leq 0.05$ or 0.01) in cerebrum length and width of mid-dose males on PND 22 (approximately 2% decrease), and a statistically significant decrease in the cerebrum length in low-dose females on PND 60 (±2) (approximately 3% decrease). However, changes were not found in the high-dose group; the findings in mid- and low-dose animals were not treatment-related. High-dose males showed a statistically-significant ($p \leq 0.01$) 3.8% decrease in linear measurements of the left parietal cortex only. No changes were noted in the right parietal cortex, and the observation is not considered biologically significant in the absence of associated brain weight, gross, or histopathological lesions.

TABLE 16. Mean brain (±SD) morphometric data [a]

Parameter	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Males				
Day 22				
Cerebrum Length (cm) Width (cm)	 1.390±0.022 1.483±0.023	 1.385±0.027 1.466±0.021	 1.365*±0.025 (2) 1.450**±0.024 (2.2)	 1.371±0.015 1.457±0.029
Cerebellum Length (cm) Width (cm)	 0.743±0.032 1.114±0.029	 0.720±0.024 1.110±0.034	 0.720±0.022 1.095±0.039	 0.730±0.035 1.103±0.032
PC Left (µm)	1595±96	-	-	1582±120
PC Right (µm)	1573±145	-	-	1589±144
FC Left (µm)	1599±105	-	-	1595±117
FC Right (µm)	1609±163	-	-	1635±100
Ncau Left (µm)	3488±219	-	-	3546±188
Ncau Right (µm)	3402±114	-	-	3316±189
Hippo Left (µm)	1665±78	-	-	1712±93
Hippo Right (µm)	1641±64	-	-	1671±108
Termination				
Cerebrum Length (cm) Width (cm)	 1.483±0.023 1.528±0.020	 1.499±0.019 1.544±0.030	 1.477±0.034 1.534±0.019	 1.483±0.024 1.512±0.030
Cerebellum Length (cm) Width (cm)	 0.776±0.043 1.160±0.026	 0.792±0.032 1.192±0.028	 0.766±0.030 1.154±0.030	 0.747±0.034 1.158±0.030
PC Left (µm)	1779±100	1783±104	1743±81	1712*±85 (3.8)
PC Right (µm)	1713±114	-	-	1692±82
FC Left (µm)	1726±71	-	-	1802±98
FC Right (µm)	1768±86	-	-	1818±93
Ncau Left (µm)	3897±150	-	-	3926±110
Ncau Right (µm)	3683±174	-	-	3649±134
Hippo Left (µm)	1836±125	-	-	1820±124
Hippo Right (µm)	1849±125	-	-	1858±106
Females				
Day 22				
Cerebrum Length (cm) Width (cm)	 1.344±0.035 1.445±0.011	 1.357±0.022 1.460±0.017	 1.365±0.041 1.461±0.026	 1.376±0.030 1.446±0.033
Cerebellum Length (cm) Width (cm)	 0.714±0.024 1.080±0.024	 0.729±0.030 1.100±0.036	 0.702±0.033 1.074±0.034	 0.724±0.029 1.088±0.037
PC Left (µm)	1590±110	-	-	1644±85
PC Right (µm)	1509±67	-	-	1516±78
FC Left (µm)	1615±62	-	-	1645±58
FC Right (µm)	1584±48	-	-	1679±91
Ncau Left (µm)	3468±186	-	-	3629±124
Ncau Right (µm)	3292±155	-	-	3389±154
Hippo Left (µm)	1628±142	-	-	1635±89

TABLE 16. Mean brain (±SD) morphometric data [a]				
Parameter	Dose (mg/kg/day)			
	0	0.01	0.08	0.15
Hippo Right (µm)	1589± 114	-	-	1616±100
Termination				
Cerebrum				
Length (cm)	1.470±0.035	1.422**±0.034 (3.3)	1.457±0.036	1.466±0.033
Width (cm)	1.497±0.015	1.494±0.020	1.506±0.022	1.497±0.020
Cerebellum				
Length (cm)	0.757±0.028	0.752±0.034	0.770±0.030	0.762±0.031
Width (cm)	1.155±0.023	1.169±0.019	1.160±0.034	1.151±0.027
PC Left (µm)	1678±70	-	-	1716±111
PC Right (µm)	1680±84	-	-	1708±89
FC Left (µm)	1715±94	-	-	1728±125
FC Right (µm)	1744±86	-	-	1755±85
Ncau Left (µm)	3692±167	-	-	3790±124
Ncau Right (µm)	3689±134	-	-	3630±135
Hippo Left (µm)	1792±84	-	-	1826±50
Hippo Right (µm)	1827±60	-	-	1808±75

[a] Data obtained from pages 298-306 in the study report. MRID 46214301.
PC Left = parietal cortex, left brain; PC Right = parietal cortex, right brain
FC Left = frontal cotex, left brain; FC Right = frontal cotex, right brain
Ncau Left = caudet nucleus, left brain; Ncau Right = caudet nucleus, right brain
Hippo = hippocampus
N = 10
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
Number in parantheses is % difference from control value calculated by reviewer

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators concluded that no clinical signs of developmental neurotoxicity were noted in male offspring at any dose levels or in low- and mid-dose female offspring. Motor activity of high-dose offspring was above the historical control values on PND 17; however, in the absence of any other clinical effects or motor activity effects on PND 22 or PND 62, the observation was not considered a sign of neurotoxicity. Dose-dependent decreases in erythrocyte and brain cholinesterase activity were noted on PND 21. The NOAEL for developmental neurotoxicity was set at 0.15 mg/kg/day, and the NOAEL for systemic toxicity was set at 0.01 mg/kg/day.

B. REVIEWER COMMENTS:

There were no treatment-related effects on mortality, clinical signs, body weight, or food consumption in dams. No treatment-related effects on reproductive parameters were observed. High-dose dams exhibited toxicologically significant inhibition of erythrocyte (26% inhibition; $p \leq 0.01$) and brain (33% inhibition; $p \leq 0.01$) cholinesterase activity on PND 21. Erythrocyte cholinesterase activity was inhibited 13% ($p \leq 0.05$) in mid-dose dams; however, this effect, while treatment-related, is not considered toxicologically significant due to the small magnitude of change. No other cholinesterase activity effects were noted in dams on PND 21.

In offspring, there were no treatment-related deaths, clinical signs or effects on birth weight, developmental landmarks, FOB parameters, auditory startle reflex, learning and memory, brain weight, brain morphology, or neuropathology. At the high dose (0.15 mg/kg/day), male and female offspring body weight was approximately 4-6% lower from LD 11 through weaning at day 21. Body weight gain was decreased for high-dose males and females during lactation days 1-11 (up to 11% decrease) and lactation days 12-13 (up to 14% decrease). No body weight effects were noted for low- or mid-dose pups during lactation. There were no toxicologically-significant post-weaning body weight effects.

There were treatment-related increases in motor activity at the mid and high dose groups. On PND 13, there were increases in males at the mid (35%) and high (58%) dose groups and in females at the mid (34%) and high (19%) dose groups. On PND 17 there were increases in males at the mid (18%) and high (26%) dose groups and in females at the mid (15%) and high (64%) dose groups.

No treatment-related changes were noted in serum, erythrocyte or brain cholinesterase activity for male or female pups on PND 4. On PND 21, significant dose-related inhibition ($p \leq 0.05$ or 0.01) of serum (56-86% decrease), erythrocyte (47-84% decrease), and brain (38-75% decrease) ChE activity were observed in mid- and high-dose males and females. No treatment-related effects were noted for low-dose animals.

The maternal LOAEL was 0.15 mg/kg/day based on inhibition of erythrocyte and brain cholinesterase activity. The maternal NOAEL was 0.08 mg/kg/day.

The offspring LOAEL was 0.08 based on increased motor activity in males and females on PND 13 and 17 and inhibition of serum, erythrocyte, brain cholinesterase activity in both sexes. The NOAEL was 0.01 mg/kg/day.

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

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

C. **STUDY DEFICIENCIES:**.

1. The following information was not provided: a basis for dosing in the definitive study (MRID 46214301); time to peak effect of cholinesterase inhibition after dosing; and positive control data.

2. The Materials and Methods section of the study report indicated that Viability and Lactation Indices would be calculated; however, the data were not presented in the results section of the report.

3. Lack of positive control data from the testing laboratory.

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R116133

Chemical:	**Terbufos**
PC Code:	**105001**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/28/2005**
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