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WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **September 13, 2005**

MEMORANDUM

TXR No.: **0050231**

SUBJECT: Tribufos - Evaluation of a Developmental Neurotoxicity

FROM: William Greear, M.P.H., D.A.B.T., Toxicologist
Registration Action Branch 1
Health Effects Division (7509C)

THROUGH: Pv Shah, Ph.D., Branch Senior Scientist
Registration Action Branch 1
Health Effects Division (7509C)

TO: Susan Lewis, Product Manager
RM-# 51
Special Review and Reregistration Division (7505C)

DP Barcode: 278790 **PC Code:** 074801

HED Conclusions: The developmental neurotoxicity study is classified as Acceptable/Non Guideline.

Action Requested: Review of a developmental neurotoxicity study in rats submitted by Bayer Corporation, Agricultural Division, Toxicology, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104.

Results of the Submitted Studies:

1. **CITATION:** Sheets, L. P. (2001) A Developmental neurotoxicity screening study with technical grade Tribufos (DEF®) in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 110523; July 27, 2001. MRID 45499501. Unpublished

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45499501), Tribufos (98.0-98.1% a.i., batch # 503-0087 5102025) was administered to parent female Wistar rats in the diet at concentrations of 0, 4, 40 or 200 ppm from gestation day 0 through postnatal day 21. The average daily intake of Tribufos was 0, 0.4, 3.4-3.5, and 16.4-18.2 mg/kg bw/day during gestation and 0, 0.6-1.0, 6.1-9.9, and 33.5-55.4 mg/kg/day during lactation, for the 0, 4, 40, and 200 ppm groups, respectively. Two studies, with a two-week difference in start date, were conducted under similar conditions. The main study (00-D72-AG) included 20 parent females/dose level, and the satellite study (00-D2-AS) included 10 parent females/dose level and was utilized to provide samples to assess cholinesterase activity. A Functional Operational Battery (FOB) was performed on 10 dams/dose from the main study on gestation days 6, 13, and 20 and on and lactation days 4, 11, and 21. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring from the main study, representing at least 20 litters/dose, were allocated for detailed clinical observations (abbreviated FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of learning and memory, electroretinography, and neuropathology at study termination (day 75 of age). Animals from the satellite study were used to provide blood and brain samples for the offspring (postnatal days 11 and 21) and dams (lactation day 21) for measurement of cholinesterase activity. Pup physical development was assessed by body weight, and sexual maturation of females was assessed by age at vaginal opening. Maturation of males was assessed by age at completion of balano-preputial separation.

No treatment-related effects were seen on survival, food consumption or reproductive parameters. Dams exhibited clinical signs of toxicity, slight tremors, at parturition which , are likely associated with the observed cholinesterase inhibition. Maternal body weight during lactation was decreased at each time point recorded for the high-dose group, with average differences of 8-12% compared to the control.

The maternal systemic LOAEL is 200 ppm (16.4 mg/kg/day) based on clinical signs (tremor) and decreased body weight during lactation. The maternal systemic NOAEL is 40 ppm (3.4 mg/kg/day).

Cholinesterase activity was decreased ($p<0.05$) in maternal animals at 40 and 200 ppm. Decreases were 66%, 76%, and 22% at 40 ppm, and 88% 87%, and 74% at 200 ppm compared to control for plasma, erythrocyte and brain cholinesterase activities, respectively.

The maternal cholinesterase LOAEL 40 ppm (3.4 mg/kg/day) based on decreases in plasma, erythrocyte and brain cholinesterase activity. The maternal cholinesterase NOAEL is 4 ppm (0.4 mg/kg bw/day).

) adverse effects on offspring survival. No treatment-related effects on body weight gains were seen at 4 or 40 ppm pups. At the high dose (200 ppm), pups erage of 16% less than controls on PND 4, 21% on PND 11 and 22% by PND 21. , PND4, high-dose pups gained 37-38% less than controls, and decreased body continued to be observed for high-dose pups throughout the lactation period. For dy weights were 21% below controls on day 27, and had partially recovered by the end dy, to approximately 12% below controls. For high-dose females, body weights were low controls on day 6, and had partially recovered by the end of the study, to ximately 8% below controls. Preputial separation for high-dose males was delayed relative ntrols. Additionally, the development of a surface-righting reflex in high dose animals was ificantly ($p \leq 0.05$) delayed. The average day of a surface righting was 6.0 days for control d 7.2 days for the high-dose animals.

Motor activity was decreased in males (15%) and females (46%) at the high dose on PND 13. Conversely, motor activity was increased in males (12%) and females (43%) at the high dose on PND 17. Changes occurred in both directions or at a lower magnitude at the low and mid dose groups for both sexes on PND 13 and PND 17. There were no apparent effects on motor activity in males or females on PND 21 and PND 60.

Startle response amplitude for high-dose males and females was decreased compared to controls on day 21, but not on subsequent test days. No treatment-related effects were seen on learning and memory. Absolute brain weights of high-dose males and females were decreased (4-13%) compared to controls on days 11 and 21. The anterior to posterior lengths of the cerebrum were marginally decreased ($p<0.05$) for high-dose males and females compared to controls on PND 11. The anterior to posterior length for the cerebellum was also marginally decreased in high-dose males and females, although the effect did not reach statistical significance

The offspring systemic LOAEL is 200 ppm (16.4 mg/kg/day), based on decreased body weight, body weight gain, delay in preputial separation, delayed surface righting reflex, decreased motor activity, decreased startle response amplitude, decreased absolute brain weights, and changes in the brain morphometrics (cerebrum and cerebellum). The offspring systemic NOAEL is 40 ppm (3.4 mg/kg/day).

For the day 21 offspring, the only effect was a 21-22% decrease in plasma cholinesterase in high-dose males and female. RBC and brain cholinesterase activity was not adversely affected in the offspring. In accordance with the current policy inhibition of plasma cholinesterase activity alone in the absence of clinical signs is not considered to be appropriate for use as the basis for a LOAEL.

The offspring cholinesterase NOAEL is 200 ppm (16.4 mg/kg/day), the highest dose tested. An offspring cholinesterase NOAEL was not established.

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

TRIBUFOS

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 45499501/0050231

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 02-18

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Disclaimer

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

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EPA Reviewer: William Greear, M.P.H., D.A.B.T.
Registration Action Branch 1, Health Effects Division (7509C)
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Signature: _____
Date _____
Signature: _____
Date _____

Template version 11/01

DATA EVALUATION RECORD
TXR#: 0050231

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

PC CODE: 074801

DP BARCODE: D278790
SUBMISSION NO.: S605415

TEST MATERIAL (PURITY): Tribufos (98.0-98.1%)

SYNONYMS: S, S, S-Tributyl phosphorotrithioate; DEF

CITATION: Sheets, L. P. (2001) A Developmental neurotoxicity screening study with technical grade Tribufos (DEF®) in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 110523; July 27, 2001. MRID 45499501. Unpublished

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorne Road, Kansas City, Missuori 64120-0013.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45499501), Tribufos (98.0-98.1% a.i., batch # 503-0087 5102025) was administered to parent female Wistar rats in the diet at concentrations of 0, 4, 40 or 200 ppm from gestation day 0 through postnatal day 21. The average daily intake of Tribufos was 0, 0.4, 3.4-3.5, and 16.4-18.2 mg/kg bw/day during gestation and 0, 0.6-1.0, 6.1-9.9, and 33.5-55.4 mg/kg/day during lactation, for the 0, 4, 40, and 200 ppm groups, respectively. Two studies, with a two-week difference in start date, were conducted under similar conditions. The main study (00-D72-AG) included 20 parent females/dose level, and the satellite study (00-D2-AS) included 10 parent females/dose level and was utilized to provide samples to assess cholinesterase activity. A Functional Operational Battery (FOB) was performed on 10 dams/dose from the main study on gestation days 6, 13, and 20 and on and lactation days 4, 11, and 21. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring from the main study, representing at least 20 litters/dose, were allocated for detailed clinical observations (abbreviated FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of learning and memory, electroretinography, and neuropathology at study termination (day 75 of age). Animals from the satellite study were used to provide blood and brain samples for the offspring (postnatal days 11 and 21) and dams (lactation day 21) for measurement of

cholinesterase activity. Pup physical development was assessed by body weight, and sexual maturation of females was assessed by age at vaginal opening. Maturation of males was assessed by age at completion of balano-preputial separation.

No treatment-related effects were seen on survival, food consumption or reproductive parameters. Dams exhibited clinical signs of toxicity, slight tremors, at parturition which , are likely associated with the observed cholinesterase inhibition. Maternal body weight during lactation was decreased at each time point recorded for the high-dose group, with average differences of 8-12% compared to the control.

The maternal systemic LOAEL is 200 ppm (16.4 mg/kg/day) based on clinical signs (tremor) and decreased body weight during lactation. The maternal systemic NOAEL is 40 ppm (3.4 mg/kg/day).

Cholinesterase activity was decreased ($p<0.05$) in maternal animals at 40 and 200 ppm. Decreases were 66%, 76%, and 22% at 40 ppm, and 88% 87%, and 74% at 200 ppm compared to control for plasma, erythrocyte and brain cholinesterase activities, respectively.

The maternal cholinesterase LOAEL 40 ppm (3.4 mg/kg/day) based on decreases in plasma, erythrocyte and brain cholinesterase activity. The maternal cholinesterase NOAEL is 4 ppm (0.4 mg/kg bw/day).

Treatment had no adverse effects on offspring survival. No treatment-related effects on body weight or body weight gains were seen at 4 or 40 ppm pups. At the high dose (200 ppm), pups weighed an average of 16% less than controls on PND 4, 21% on PND 11 and 22% by PND 21. From birth to PND4, high-dose pups gained 37-38% less than controls, and decreased body weight gain continued to be observed for high-dose pups throughout the lactation period. For males, body weights were 21% below controls on day 27, and had partially recovered by the end of the study, to approximately 12% below controls. For high-dose females, body weights were 20% below controls on day 6, and had partially recovered by the end of the study, to approximately 8% below controls. Preputial separation for high-dose males was delayed relative to controls. Additionally, the development of a surface-righting reflex in high dose animals was significantly ($p \leq 0.05$) delayed. The average day of a surface righting was 6.0 days for control and 7.2 days for the high-dose animals.

Motor activity was decreased in males (15%) and females (46%) at the high dose on PND 13. Conversely, motor activity was increased in males (12%) and females (43%) at the high dose on PND 17. Changes occurred in both directions or at a lower magnitude at the low and mid dose groups for both sexes on PND 13 and PND 17. There were no apparent effects on motor activity in males or females on PND 21 and PND 60.

Startle response amplitude for high-dose males and females was decreased compared to controls on day 21, but not on subsequent test days. No treatment-related effects were seen on learning and memory. Absolute brain weights of high-dose males and females were decreased (4-13%) compared to controls on days 11 and 21. The anterior to posterior lengths of the cerebrum were

marginally decreased ($p<0.05$) for high-dose males and females compared to controls on PND 11. The anterior to posterior length for the cerebellum was also marginally decreased in high-dose males and females, although the effect did not reach statistical significance

The offspring systemic LOAEL is 200 ppm (16.4 mg/kg/day), based on decreased body weight, body weight gain, delay in preputial separation, delayed surface righting reflex, decreased motor activity, decreased startle response amplitude, decreased absolute brain weights, and changes in the brain morphometrics (cerebrum and cerebellum). The offspring systemic NOAEL is 40 ppm (3.4 mg/kg/day).

For the day 21 offspring, the only effect was a 21-22% decrease in plasma cholinesterase in high-dose males and female. RBC and brain cholinesterase activity was not adversely affected in the offspring. In accordance with the current policy inhibition of plasma cholinesterase activity alone in the absence of clinical signs is not considered to be appropriate for use as the basis for a LOAEL.

The offspring cholinesterase NOAEL is 200 ppm (16.4 mg/kg/day), the highest dose tested. An offspring cholinesterase NOAEL was not established.

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: Tribufos

Description:	Clear, colorless liquid
Lot/Batch #:	503-0087 5102025
Purity:	98.0-98.1 % a.i.
Compound Stability:	Confirmed for 8 months
CAS # of TGAI:	78-48-8
	$(CH_3CH_2CH_2CH_2S)_3P=O$

2. Vehicle and/or positive control: corn oil in the diet

3. Test animals (P):

Species:	Rat	
Strain:	Wistar Crl:W(HAN)BR	
Age at study initiation:	females: at least 12 wks; males: at least 15 weeks (breeders only)	
Wt. at study initiation:	149.6-229.7 g	
Source:	Charles River Laboratories	
Housing:	Individually or with litter in stainless steel grid or plastic cages	
Diet:	Purina Mills Rodent Lab Chow 5001-4, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	Not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: January 2, 2000; End: April, 2000

2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day 0 through lactation day 21. Pups were weaned on postnatal day 21, after which time maternal animals were killed. Two studies, with a two-week difference in start date, were conducted under similar conditions. The main study (00-D72-AG) included 20 parent females/dose level, and the satellite study (00-D2-AS) included 10 parent females/dose level and was utilized to provide samples to assess cholinesterase activity. F1 pups remained on study up to postnatal days 70-80 (study termination for main study).

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

individual cage with a solid bottom and bedding, where the dam was maintained through gestation and lactation.

4. **Animal assignment:** Mated females and offspring were allocated as shown in Table 1 using an animal allocation program written in SAS. For offspring, four sets of animals (designated sets A-D) were utilized for assessment at each age. Randomly-selected pups (10/sex/dose) were designated as Set D and were perfused with fixative and brains were collected for histopathological examination and morphometric analysis; however, animal identification was abraded from the labels; therefore, it was necessary to use pups from the satellite study for these parameters.

   Sixteen pups/sex/group from the main study were allocated on postnatal day 4 to each of the following: motor activity, acoustic startle habituation, passive avoidance, water maze detailed observational battery, and sacrifice and brain examination on postnatal day 21. At approximately 50-60 days of age, a minimum of 10 offspring/sex/dose level were given an opthalmoscopic examination. On day 70-80, electroretinography was performed, animals were sacrificed by perfusion and brain weights recorded.

TABLE 1. Study design

Experimental parameter		Dose (ppm in diet)			
		0	4	40	200
Maternal animals–Main study					
		No. of maternal animals assigned			
FOB (GD 6, 13, 20; LD 4, 11, 21)		10	10	10	10
Offspring– Main study					
Set A	Motor activity (PND 13, 17, 21, 58-62)	16/sex	16/sex	16/sex	16/sex
Set B	Acoustic startle habituation (PND 22, 36-40, 58-62)	16/sex	16/sex	16/sex	16/sex
Set C	Passive Avoidance (PND 22, 29)	16/sex	16/sex	16/sex	16/sex
	Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	16/sex	16/sex	16/sex	16/sex
	Water maze (PND 58-62, 7 days after first test)	16/sex	16/sex	16/sex	16/sex
Sets A-C	Opthalmologic evaluation (PND 50-60)	10/sex	10/sex	10/sex	10/sex
	Electroretinography (PND 70-80)	10/sex	10/sex	10/sex	10/sex
	Brain Weight (PND 70-80)	10/sex	10/sex	10/sex	10/sex
Maternal animals-Satellite study					
Erythrocyte, Plasma, and Brain Cholinesterase Activity (LD 21)		10	10	10	10
Offspring- Satellite study					
Erythrocyte, Plasma, and Brain Cholinesterase Activity (PND 11, 21)		10/sex	10/sex	10/sex	10/sex
Set D	Gross Necropsy and Brain Measurements (PND 11, 21)	9-12/sex	9-12/sex	9-12/sex	9-12/sex

5. **Dose selection rationale:** Dose levels were chosen based on the results from a two-generation reproduction study in Sprague-Dawley rats (Report 101255; MRID 42040201). In that study, Tribufos was administered in the diet at levels of 0, 4, 32, and 260 ppm

beginning 10 weeks before mating. Signs of toxicity in the 260 ppm first generation offspring included increased cannibalization, increased incidence of litters with stillborn pups, decreased pup viability, and decreased pup body weight gain during lactation. Dams exhibited decreased body weight gain at 260 ppm. Dose-related decreases in plasma, erythrocyte and brain cholinesterase were observed in both dams and offspring, and were biologically significant at 32 and 260 ppm. Based on these results, the doses selected for the developmental neurotoxicity study were 0, 4, 40, and 200 ppm. The 200 ppm level was selected to produce evidence of toxicity and approximate a MTD. The 4 ppm dose was selected to produce no signs of toxicity and no cholinesterase inhibition, and the 40 ppm level was selected as an intermediate dose to assist in establishing compound-related effects and a NOAEL.

6. **Dosage administration:** Tribufos was administered to parent female Wistar rats in the diet at levels of 0, 4, 40 or 200 ppm from gestation day 0 through postnatal day 21. The test substance intake was 0, 0.4, 3.4-3.5, and 16.4-18.2 mg/kg bw/day, respectively, for analytically-determined concentrations of 0, 4.2, 20, and 209 ppm in the diet during gestation. The test substance intake was 0, 0.6-1.0, 6.1-9.9, and 33.5-55.4 mg/kg bw/day, respectively, for analytically-determined concentrations of 0, 4.2, 40, and 209 ppm in the diet during lactation.
7. **Dosage preparation and analysis:** Detailed descriptions of feed preparations and test diet analysis were not provided; however, information from the study report is as follows. Corn oil was used as the vehicle for the test article at 1% by weight of the diet, and acetone served as a solvent in the diet preparation process and was allowed to evaporate. Concentrations of the test substance in the diet were measured by gas chromatography four times during the in-life phase of the two studies. Homogeneity and stability data from a previous study (utilizing concentrations of 2, 4, and 600 ppm) were presented.

Results:

Homogeneity analysis: was not performed for this study; however the study report states, "At nominal concentrations of 4 ppm and 600 ppm, tribufos is homogeneously distributed and stable for at least 10 days at room temperature and 28 days at freezer storage conditions."

Stability analysis: was not performed for this study; however the study report states, "At nominal concentrations of 4 ppm and 600 ppm, tribufos is homogeneously distributed and stable for at least 10 days at room temperature and 28 days at freezer storage conditions."

Concentration analysis: The mean analytical concentrations of test solutions were 0, 4.2, 40, and 209 ppm for the main study, and 0, 4.0, 42, and 214 ppm for the satellite study, respectively, for the 0, 4, 40, and 200 ppm diets.

The analytical data indicated the concentration of Tribufos in the diets was adequate. However, the use of stability and homogeneity data from a previous study is questionable.

C. OBSERVATIONS

1. **In-life observations:**

a. **Maternal animals:** Once daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals.

Ten dams per group were observed (by observers blind to the treatment group) outside the home cage during the gestation dosing period (days 6, 13 and 20) and during the lactation dosing period (days 4, 11 and 21). The following functional observations were recorded.

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

Individual maternal body weight and food consumption data were recorded weekly for gestation days 0-6, 6-13, 13-20, and lactation days 0-7, 7-14, and 14-21.

From gestation day 20, dams were checked daily for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Numbers of live and dead offspring were recorded during parturition.

b. **Offspring:**

1. **Litter observations:** 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); excess pups were killed and discarded.

2. **Developmental landmarks:** Beginning on postnatal day 38, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset was recorded.

3. **Detailed observations:** Offspring were examined for clinical signs once daily during the preweaning period and once weekly after weaning by observers blind to the treatment groups. Individual offspring body weight data were recorded on postnatal days 0, 4, 11, 17, and 21

and once weekly thereafter. Individual food consumption was measured weekly from the week of postnatal day 28.

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

5. **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, a total of 16 offspring/sex/group (one male or one female from each litter) was examined outside the home cage in an FOB assessment by observers blind to the treatment groups. On postnatal days 4 and 11, the animals were not evaluated in the open field, unless deemed necessary by the observer. Otherwise, methods were similar to the procedures used for the dams.

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

6. **Motor activity testing:** Motor activity was evaluated in 13-16 rats/sex/dose on days 13, 17, 21, and 60. Animals were placed individually in figure-eight mazes and were continuously monitored over a 1-hour period. An automated activity monitoring system collected data over successive 10-minute intervals by recording infra-red light source break frequency within the maze. Motor activity was measured as the number of beam interruptions that occurred during the test session, and locomotor activity was measured by eliminating consecutive counts for a given beam. Therefore, only one interruption of a given beam was counted for locomotor activity until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrease in activity over consecutive test-session intervals.

7. **Auditory startle habituation:** Auditory startle reflex habituation was performed on 16 offspring/sex/dose on postnatal days 22, 38 and 60, using an automated system.

   Animals were acclimated for 5 minutes to background noise and were then presented with the startle stimulus at 10-second intervals. The startle stimulus consisted of 50-millisecond bursts of white noise at approximately120 dB. Peak response amplitude (g force exerted on

the platform) and latency (msec) measurements were recorded for each animals' individual response curve. Response amplitude was defined as the maximum value of the average curve minus the baseline (body weight). Latency to peak was defined as the time, in msec, following onset of the stimulus when the peak response amplitude occurred.

8. Learning and memory testing:

PASSIVE AVOIDANCE CONDITIONING: On postnatal days 24 and 31, learning and short- and long-term retention were assessed in a passive avoidance test of 16 offspring/sex/dose. Testing was done in individual isolation cubicles each with a single shuttle cage. Each cubicle was insulated to attenuate sound and had a fan for ventilation. Each 7 x 7 inch shuttle cage was separated into two equal-sized compartments by a centrally-located sliding door. The two compartments were identical except that the walls in one compartment were lined with black film (dark side) and the walls in the other compartment were not lined and this compartment was illuminated with a high-intensity lamp. The lamp was switched on at the beginning of each trial and remained on until the rat crossed into the dark compartment or the trial ended. The cage floor was constructed of a stainless steel grid and the movement of the rat from the light to dark side was detected by a photocell. Rats were placed individually into the shuttle cage facing toward the light. After 20 seconds, the light was switched on and the door separating the compartments was opened. When the rat crossed into the dark side, the door closed, a brief, mild shock (0.5 sec, 0.5mA) was delivered, and the light was switched off. If the rat failed to cross to the dark side within 180 seconds, it was returned to the holding cage and assigned a latency time of 180 sec. The procedure was repeated until the rat either remained in the bright side for 180 seconds for two consecutive trials or until 15 trials had elapsed (whichever occurred first). Rats that failed to reach criterion performance within 15 trials or failed to cross during the first two acquisition trials were excluded from the retention phase of the experiment.

WATER MAZE: Learning and memory testing was performed in 16 offspring/sex/dose on postnatal days 60 and again seven days later using an —water maze. Only rats that demonstrated acquisition on the first test occasion were tested for retention seven days later. The water maze was made of opaque Plexiglas with 5-inch wide corridors. The walls were 16-inches high with approximately 7.5 inches of water. The maze was filled with water at 22±1°C. For each test trial, the rat was placed at the base of the —maze stem, between the two lateral arms. On the learning trial (first trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and was then removed from the maze. The initial arm chosen on the learning trial was designated the incorrect goal during the subsequent trials (15 maximum). Rats failing to make a correct goal choice within 60-seconds in any given trial were led to the correct goal with the exit ramp and then removed from the water. The inter trial interval was approximately 15 seconds. Each rat was required to reach a criterion of 5 consecutive error-less trials to stop the test session. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as well as the number of errors (incorrect turns) during each trial.

9. Opthalomology and Electroretinography

At postnatal days 50-60, indirect opthalmoscopy was performed on 9-10 offspring/sex/dose (that had been selected for perfusion) following dilation with a mydriatic agent.

On postnatal day 75, the same 10 offspring/sex/dose were also tested by electroretinography because retinal degeneration had been observed in adult rats at termination in a previously conducted chronic toxicity/oncogenicity study. Testing took place in a semi-dark room using commercial equipment to elicit an adequate ERG waveform, utilizing a single flash maximal response stimulus.

10. Postmortem observations:

Cholinesterase measurements

Blood and brain samples were collected from 10 dams/dose group (from the satellite study) on lactation day 21 and from 1 pup/sex/litter (from the satellite study) on postnatal days 11 and 21. Blood samples were collected from the orbital plexus of dams and offspring on postnatal day 21 and by decapitation for offspring on postnatal day 11 for determination of plasma and erythrocyte cholinesterase activities. Brain tissues were collected immediately after blood collection for the determination of brain cholinesterase activity. Post natal day 11 was included to establish levels of cholinesterase inhibition during perinatal development, when the only exposure route for the offspring was through the milk. Postnatal day 21 was chosen to represent the time of peak cholinesterase inhibition for pups due to combined exposure through milk and treated diet.

a. **Maternal animals:** Maternal animals were sacrificed by carbon dioxide inhalation on postnatal day 21. Adult females were not routinely subjected to a gross necropsy. Maternal animals found moribund were sacrificed. Those found moribund or dead were subjected to a macroscopic necropsy, with possible collection of tissues at the discretion of the study director.

b. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 11, 21 or 70-80. These animals were subjected to postmortem examinations as described below.

At postnatal day 11, 9-12 pups/sex/group were selected for gross necropsy and brain weight measurements. Animals were sacrificed by intraperitoneal injection of Fatal Plus and underwent a gross necropsy. The calivaria were sliced at the top of the skull to expose the brain and the entire head was immersed in 10% buffered formalin for 24 hours. The brain with olfactory bulbs was removed and weighed. Anterior to posterior cerebrum and cerebellum length were measured by a technician not blind to treatment using a Vernier caliper.

At postnatal day 21, up to 9-12 pups/sex/group were sacrificed by intraperitoneal injection of barbiturate and perfused via the left ventricle with a sodium nitrite flush followed by fixation

with gluteraldehyde and paraformaldehyde. The brain was collected, weighed, and post-fixed with 10% buffered formalin for 24 hours. Anterior to posterior cerebrum and cerebellum length were measured by an individual not blind to treatment using a Vernier caliper. Brains from all dose groups were embedded in paraffin and were sectioned for control and high-dose animals. Tissues were sectioned at 5 µm and stained with hematoxylin and eosin, luxol fast blue/cresyl violet and Sevier-Munger stains. Eight coronal sections from control and high-dose animals were examined microscopically.

The following brain morphometric measurements were performed:

Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm)

Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)

Caudate putamen and underlying globus pallidus diagonal width (coronal section taken at the level of the optic chiasm)

Corpus callosum (thickness at the midline)

Hippocampal gyrus (greatest dorsal-ventral thickness)

Cerebellum (roof of the fourth ventricle to the dorsal surface)

On postnatal day 70-80, 10 animals/sex/group were euthanized by carbon dioxide asphyxiation, underwent a gross necropsy and the brains were removed and weighed (fresh weight) and discarded. Another 10 rats/sex/dose were sacrificed by intraperitoneal injection of barbiturate and perfused via the left ventricle with a sodium nitrite flush followed by fixation with gluteraldehyde and paraformaldehyde. The brain, spinal cord, both eyes with optic nerves, peripheral nerves, gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected, weighed (brain only), and post-fixed with 10% buffered formalin for 24 hours. Anterior to posterior cerebrum and cerebellum length were measured by an individual not blind to treatment using a Vernier caliper.

The following central and peripheral nervous tissues were dissected and preserved in paraffin (CNS tissues) or plastic (PNS tissues): eight coronal sections of the brain, cervical, thoracic, and lumbar sections of the spinal cord, the cauda equina, eyes, optic nerves, gastrocnemius muscle, dorsal root ganglia and fibers, and gasserian ganglion. Tissues from all dose groups were embedded; however, only control and high-dose tissues were examined unless effects warranted examination of low- and mid-dose samples. Paraffin-embedded tissues were sectioned at 5 µm and stained with hematoxylin and eosin. Plastic-embedded tissues were sectioned at 2-3 µm and stained with a modified Lee's stain.

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

Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm)

Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)

Caudate putamen and underlying globus pallidus diagonal width (coronal section taken at the level of the optic chiasm)

Corpus callosum (thickness at the midline)

Hippocampal gyrus (greatest dorsal-ventral thickness)

Cerebellum (roof of the fourth ventricle to the dorsal surface)

D. DATA ANALYSIS

1. **Statistical analyses:** Continuous data were initially analyzed for equality of variance using Bartlett's test. Group means with equal variances were further analyzed with ANOVA, followed by Dunnett's test if significance was identified with the ANOVA. Group means with unequal variances were analyzed by Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons. The level of significance was set at $p \leq 0.05$, except for Bartlett's test which was set at $p \leq 0.001$.

   Motor and locomotor activity were analyzed with ANOVA, followed by Dunnett's test if significance was attained with ANOVA. Acoustic startle peak amplitude data were analyzed by ANOVA followed by Dunnett's test if significance was observed with the ANOVA. The response amplitude data for each block of 10 trials were subjected to a Repeated-Measures ANOVA, using the test block as the repeated measure. Passive avoidance latency data were analyzed with a Wilcoxon Test for time to failure. The number of trials to criterion were analyzed with Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's exact test for retention. The number of rats failing to meet the criterion level of performance in the acquisition phase was treated as incidence data. Water maze data were analyzed by a univariate ANOVA followed by Dunnett's test. The number of trials to criterion and the number of errors were analyzed with Kruskal-Wallace and Wilcoxon test for the acquisition phase and Fischer's exact test for retention. The number of rats failing to meet the criterion level of performance in the water maze learning phase was treated as incidence data. Micropathology frequency data were analyzed by Chi-Square followed by Fisher's Exact Test if significance was identified with the Chi-Square.

2. **Indices:**

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

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

Mating index = (Number of inseminated females/Number of females co-housed with males) × 100

Fertility index = (Number of pregnant females/Number of inseminated females) × 100

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

Birth index = (Total No. of offspring born/Total No. of implantation sites) × 100

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

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

Lactation index = (Number of live offspring on Day 21/Number of live offspring on PND 4 after culling) × 100

3. **Positive and historical control data:**

Positive control have been submitted by the testing laboratory and are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** There were no maternal deaths before scheduled termination, and there were no treatment-related clinical signs observed during gestation. Slight tremors were observed in five high-dose dams on the day of parturition, and are likely associated with the observed cholinesterase inhibition (described below). There were no other clinical signs observed during lactation.

There were no toxicologically significant substance-related functional observations during gestation or lactation. However, on gestation day 6, high-dose dams exhibited a decrease ($p \leq 0.05$) in the average number of rearings compared to controls. This effect is biologically questionable due to rather large standard deviations.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 2. There were no significant treatment-related effects on body weight or body weight gain during gestation. Body weight during lactation was decreased at each time point recorded for the high-dose group, with average differences of 8-12% compared to the control. A decrease ($p \leq 0.05$), considered incidental to treatment, was also observed for low-dose animals on lactation day 21.

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

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

Observations/study interval	Dose (ppm)			
	0	4	40	200
Gestation (n= 26-29)				
Body wt. Gestation day 0 (g)	202.6±2.83	198.4±2.95	200.2±2.86	200.0±2.42
Body wt. Gestation day 6 (g)	223.6±2.81	219.7±2.74	222.2±2.66	218.9±2.51
Body wt. Gestation day 13 (g)	248.1±2.99	243.7±3.15	248.8±3.25	241.9±3.15
Body wt. Gestation day 20 (g)	302.7±5.81	306.0±3.96	310.4±5.43	291.8±4.55
Wt. gain gestation days 6-20 (g)	100.1±4.96	107.6±2.66	110.2±3.69	91.8±3.48
Food consumption gestation days 0-6 (g/kg/day)	84.1±1.13	86.8±2.89	84.4±1.09	78.4±1.50
Food consumption gestation days 6-13 (g/kg/day)	87.6±1.16	89.3±1.86	88.1±1.09	86.9±1.51
Food consumption gestation days 13-20 (g/kg/day)	81.2±2.37	86.2±1.37	87.0±0.91	84.0±2.29
Lactation (n=19-29)				
Body wt. lactation day 0(g)	240.9±4.20	241.2±2.97	245.8±3.78	216.8±3.05*
Body wt. lactation day 4 (g)	254.8±3.60	252.4±3.46	263.0±4.24	235.3±3.74**
Body wt. lactation day 7 (g)	261.6±3.32	258.4±3.33	268.8±3.95	241.8±2.99**
Body wt. lactation day 14(g)	279.7±3.73	272.9±3.52	284.1±4.01	246.8±3.07**
Body wt. lactation day 21(g)	284.4±3.17	271.0±3.42*	280.3±3.51	254.6±3.55**
Food consumption lactation days 0-7 (g/kg/day)	149.3±8.65	146.0±15.29	153.6±13.57	160.3±10.62
Food consumption lactation days 7-14 (g/kg/day)	204.2±9.43	206.9±15.44	210.4±14.27	183.7±8.74
Food consumption lactation days 14-21 (g/kg/day)	238.0±6.20	236.3±6.76	247.7±12.84	264.9±26.98

[a] Data obtained from Tables 3 & 4 pages 68-71 and Tables 6 & 7 pages 74-77, MRID 45499501.

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. The only treatment-related effect was a decreased gestation index for the high-dose animals compared to controls. Although the difference was not statistically significant, the authors considered it treatment-related based on the magnitude of difference, occurrence at the high-dose and other evidence of toxicity at this dose in this study, and pup lethality and decreased viability in a reproductive toxicity study at 260 ppm.

TABLE 3. Reproductive Performance [a]				
Observation	Dose (ppm)			
	0	4	40	200
Number Mated	30	30	30	30
Number Delivered	23	23	21	19
Mating Index (%)	100	100	100	100
Fertility Index (%)	90.0	96.7	86.7	93.3
Gestation index (%)	85.2	79.3	80.8	67.9

[a]Data obtained from Table 1, pages 64-65, MRID 45499501.

4. **Maternal postmortem results:** Results for the cholinesterase studies in dams are presented in Table 15 below, along with the results for the offspring. For dams, a dose-related decrease in cholinesterase activity was noted at 40 and 200 ppm. Percent inhibition compared to controls was as follows:
   Plasma: 3% at 4 ppm, 66% at 40 ppm, and 88% at 200 ppm
   Erythrocyte: 14% at 4 ppm, 76% at 40 ppm, and 87% at 200 ppm
   Brain: 2% at 4 ppm, 22% at 40 ppm, and 74% at 200 ppm

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 4. There was no treatment-related effect on the number of litters, live litter size, number of stillborn pups, live birth index, or viability index.

TABLE 4. Litter size and viability [a]				
Observation	Dose (ppm)			
	0	4	40	200
Number of Litters	23	23	21	19
Total number born	252	261	237	220
Number born live	251	261	236	218
Number born dead	1	0	1	2
Mean No. of viable pups:				
Day 0	11	11	11	12
Day 4 [b]	11	11	11	11
Day 4 [c]	8	8	8	8
Day 21	8	8	8	8
Live birth index (%)	99.6	100	99.6	99.0
Viability index	97.3	99.6	99.2	99.2
Lactation index	100	99.5	99.4	96.1

[a]Data obtained from Table 9, pages 81-83, MRID 45499501.
[b]Before standardization (culling).
[c]After standardization (culling).

2. **Body weight:** Body weights were comparable at birth across all dose groups; however, by PND 4, high-dose pups weighed an average of 16% less than controls. At PND 11, high-dose

pups weighed 21% less than controls and by day 21, high-dose pups weighed 22% less than controls. No body weight effects were noted at 4 or 40 ppm. Body weight gain was also decreased in high-dose pups. From birth to PND4, high-dose pups gained 37-38% less than controls, and decreased body weight gain continued to be observed for high-dose pups throughout the lactation period. No treatment-related effects on body weight gain were noted in low- or mid-dose pups. Selected mean preweaning pup body weight data are presented in Table 5.

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

Postnatal Day	Dose (ppm)							
	0	4	40	200	0	4	40	200
	Males				Females			
0	5.8±0.16	5.9±0.09	5.8±0.08	5.6±0.13	5.4±0.15	5.5±0.09	5.6±0.07	5.3±0.11
4[b]	9.3±0.31	9.1±0.18	9.4±0.16	7.8*±0.31 (16%)[d]	8.8±0.28	8.8±0.17	9.1±0.14	7.4*±0.28 (16%)[d]
4[c]	9.3±0.31	9.1±0.19	9.4±0.16	7.8*±0.31 (16%)[d]	8.9±0.28	8.8±0.17	9.0±0.15	7.4*±0.29 (17%)[d]
11	22.2±0.56	21.5±0.39	22.3±0.32	17.6*±0.67 (21%)[d]	21.5±0.48	20.7±0.46	21.7±0.37	17.1*±0.64 (20%)[d]
17	33.9±0.58	32.3±0.56	33.2±0.57	26.2*±0.69 (23%)[d]	32.7±0.49	31.3±0.61	32.1±0.57	25.5*±0.66 (22%[d])
21	43.2±0.79	41.4±0.84	42.8±0.70	33.6*±0.96 (22%)[d]	41.8±0.65	40.3±0.90	41.3±0.71	32.6*±0.93 (22%)[d]
Weight gain Days 0-4	3.7±0.18	3.4±0.14	3.6±0.10	2.3*±0.23 (38%)[d]	3.5±0.17	3.4±0.13	3.6±0.09	2.2*±0.23 (37%)[d]
Weight gain Days 4-11	13.0±0.29	12.3±0.29	13.0±0.25	9.6*±0.50 (26%)[d]	12.7±0.26	11.8±0.36	12.6±0.34	9.4*±0.49 (26%)[e]
Weight gain Days 4-17	24.7±0.37	23.1*±0.46	23.8±0.52	18.2*±0.53 (26%)[d]	23.9±0.33	22.5±0.53	23.1±0.56	17.8*±0.52 (26%)[e]
Weight gain Days 4-21	34.0±0.52	32.3±0.72	33.4±0.67	25.6*±0.77 (25%)[d]	33.0±0.44	31.4±0.80	32.3±0.70	25.0*±0.78 (24%)[e]

[a] Data obtained from Tables12-13, pages 88-96, MRID 45499501. *p<0.05.
[b] Before standardization (culling).
[c] After standardization (culling).
[d] (%) decrease compared to controls, calculated by reviewer

Body weights were decreased in high-dose males and females compared to controls following weaning. For males, body weights were 21% below controls on day 27, and had partially recovered by the end of the study, to approximately 12% below controls. For high-dose females, body weights were 20% below controls on day 6, and had partially recovered by the end of the study, to approximately 8% below controls. No biologically-significant postweaning body weight effects were noted at 4 or 40 ppm. Although there were some statistically significant differences noted at 4 and 40 ppm, the lower body weights at the low- and mid-doses are not considered toxicologically significant due to the small magnitude of change and sporadic incidence. Selected mean postweaning offspring body weight data are presented in Table 6.

TABLE 6. Mean (±SD) post-weaning pup body weights (g) [a]								
Postnatal Day	Dose (ppm)							
	0	4	40	200	0	4	40	200
	Males				Females			
27	69.1±7.3	65.7*±7.1 (5%)	68.9±6.7	54.3*±7.8 (21%)	69.8±6.4	66.9*±6.7 (4%)	68.6±6.3	55.7*±7.4 (20%)
34	115.2±11.1	109.6*±10.3 (5%)	114.3±9.3	92.3*±12.9 (20%)	107.0±8.6	102.9*±8.7 (4%)	105.0±8.1	89.1*±10.1 (17%)
41	162.4±15.0	152.5*±14.2 (6%)	154.2*±14.7 (5%)	133.7*±14.5 (18%)	134.5±9.9	130.9±9.8	133.3±10.4	117.3*±10.1 (13%)
48	203.8±18.1	196.7*±15.8 (3.5%)	203.0±15.6	173.2*±18.3 (15%)	154.9±11.9	151.0±11.3	151.4±12.1	135.6*±11.8 (12%)
55	246.8±21.3	239.8±19.2	244.8±19.1	212.5*±21.4 (14%)	169.6±16.1	168.5±12.7	168.7±15.6	156.3*±11.5 (8%)
62	280.9±22.7	273.9±20.9	280.6±19.4	246.4*±20.5 (12%)	184.0±16.4	179.7±13.2	181.7±13.9	168.7*±11.8 (8%)
69	309.5±25.7	300.2±23.8	306.7±21.4	273.2*±22.8 (12%)	194.1±18.2	188.1±15.9	191.6±16.3	178.4*±13.2 (8%)

a Data obtained from Table15, pages 99-101, MRID 45499501. *p<0.05
Number in parentheses = % decrease compared to controls, calculated by reviewer

Food consumption (untreated feed) was decreased in high-dose males and females compared to controls during the first two weeks after weaning. The decrease was 11-19% for males and 8% for females. No other postweaning food consumption effects were noted.

3. Developmental landmarks:

a. Sexual maturation: The data are presented in Table 7a. Preputial separation for high-dose males was delayed relative to controls. There were no treatment-related effects on the mean age for attainment of vaginal opening for females.

TABLE 7a. Mean (±SD) age of sexual maturation (days) [a]				
Parameter	Dose (ppm)			
	0	4	40	200
N (M/F)	23/23	23/23	21/21	19/19
Preputial separation (males)	44.3±0.26	44.4±0.29	45.0±0.44	46.5**±0.50
Vaginal opening (females)	34.6±0.43	35.0±0.40	34.7±0.31	35.0±0.51

a. Data obtained from Table 14, pages 97-98, MRID 45499501. **p<0.01

b. Surface Righting:

The data are presented in Table 7b. The development of a surface righting reflex in high-dose animals was marginally delayed (p<0.05). The average day of surface righting was 6.0 days for controls and 7.2 days for high-dose animals. No other effects were noted.

TABLE 7b. Mean (±SD) age of sexual maturation (days) [a]				
Parameter	Dose (ppm)			
	0	4	40	200
N	23	23	21	19
Mean	6.0	6.1	6.1	7.2 *
S.E	0.16	0.15	0.19	0.39
Pup reaching criteria %	100	100	100	93

[a] Data obtained from Table 14, page -98, MRID 45499501 *p<0.01

4. Behavioral assessments:

a. Functional observational battery: There were no treatment-related effects at any dose level on any test day (PND 4, 11, 21, 35, 45, or 60).

b. Motor activity: Total activity data are presented in Tables 8 and 9. Motor activity was decreased in males (15%) and females (46%) at the high dose on PND 13. Conversely, motor activity was increased in males (12%) and females (43%) at the high dose on PND 17. Changes occurred in both directions or at a lower magnitude at the low and mid dose groups for both sexes on PND 13 and PND 17. There were no apparent effects in male or females on PND 21 and PND 60. Table 9 shows that PND 13 males had decreased (75%) motor activity at the high dose. There were no other effects observed.

TABLE 8. Mean (±S.D.) motor activity data (total activity counts for session) [a]				
Test Day	Dose (ppm)			
	0	4	40	200
Males				
PND 13	141±103	91±77	128±135	120±107 (↓15%)
PND 17	338±189	305±247	301±139	383±244 (↑12%)
PND 21	481±166	450±227	466±231	462±209
PND 60	612±120	624±128	627±171	653±178
Females				
PND 13	85±90	126±130	96±91	46±55 (↓46%)
PND 17	231±164	286±178	249±129	330± 241 (↑43%)
PND 21	459±129	436±180	385±144	428±178
PND 60	803±265	860±236	774±206	947±527

[a] Data obtained from Table 19, pages 203-205, MRID 45499501
N = 13-16/sex/dose A system failure resulted in the loss of data for a total of 16 animals on PND 21 (3 control, 3 low-dose, 1 mid-dose, and 3 high-dose males; and 1 control, 2 low-dose, 2 mid-dose, and 1 high-dose females).

TABLE 9. Mean (±S.D.) locomotor activity data (total activity counts for session) [a]

Test Day	Dose (ppm)			
	0	4	40	200
Males				
PND 13	20±26	10±9	22±29	5±5 (↓75%)
PND 17	79±46	58±49	56±38	74±46
PND 21	116±46	99±40	98±41	107±43
PND 60	410±116	413±104	397±144	390±84
Females				
PND 13	10±19	16±26	15±24	7±11
PND 17	53±41	57±30	56±30	75±61
PND 21	124±43	102±33	97±32	110±45
PND 60	439±137	462±117	431±175	526±344

[a] Data obtained from Table 20, pages 206-208, MRID 45499501
N = 13-16/sex/dose. A system failure resulted in the loss of data for a total of 16 animals on PND 21 (3 control, 3 low-dose, 1 mid-dose, and 3 high-dose males; and 1 control, 2 low-dose, 2 mid-dose, and 1 high-dose females).

c. **Auditory startle reflex :** Startle response amplitude for high-dose males and females was decreased compared to controls on day 22, but not on subsequent test days. Habituation was evident in control males and females on all test days as a decrease in response amplitude over the test session. There were no effects on startle response amplitude at lower doses and there was no treatment -related effect noted for peak latency at any dose on any test day. Peak amplitude data are summarized in Table 10 and latency data are summarized in Table 11.

TABLE 10. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	4	40	200
Males					
PND 22	1	40±20	36±16	42±23	23*±9
	2	39±21	29±13	34±19	22*±8
	3	34±17	27±12	30±16	19*±5
	4	32±15	28±14	29±15	19*±8
	5	31±18	27±13	30±18	17±8
	Mean	35±17	29±13	33±17	20*±7
PND 38	1	86±35	95±45	115±67	64±34
	2	77±49	83±47	88±53	60±38
	3	65±38	75±45	85±52	53±17
	4	54±27	68±45	66±42	51±32
	5	57±21	56±33	64±39	45±21
	Mean	68±31	75±39	84±49	55±23
PND 60	1	278±117	296±159	263±132	206±132
	2	233±165	251±146	257±196	184±107
	3	215±128	203±136	213±150	163±99
	4	169±94	169±120	181±131	120±61
	5	141±58	147±104	147±79	95±43

TABLE 10. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	4	40	200
	Mean	207±102	213±124	212±132	153±79
		Females			
PND 22	1	36±13	40±17	36±10	22*±12
	2	33±13	33±12	31±9	19*±13
	3	30±10	30±15	30±8	16*±7
	4	27±7	27±11	29±9	14*±7
	5	26±8	24±8	26±10	15*±7
	Mean	30±8	31±11	30±7	17*±8
PND 38	1	74±39	75±54	61±24	52±21
	2	60±33	62±49	50±20	41±18
	3	51±33	57±39	37±12	35±15
	4	48±20	47±31	43±19	40±14
	5	45±23	40±25	45±26	36±17
	Mean	56±27	56±35	47±17	41±15
PND 60	1	104±57	99±40	82±29	98±51
	2	102±73	87±39	72±25	68±31
	3	73±33	75±37	61±24	77±43
	4	66±33	69±37	54±24	65±37
	5	59±28	63±34	46±19	75±37
	Mean	81±41	79±32	64±19	78±33

[a]Data obtained from Tables 23-24, pages 227-236, MRID 45499501
N = 16/sex/dose

TABLE 11. Auditory startle latency to peak data (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	4	40	200
Males					
PND 22	1	39±5	40±7	38±7	42±6
	2	41±9	39±8	39±8	38±7
	3	38±8	38±7	39±8	39±8
	4	39±6	41±9	38±8	39±4
	5	38±7	38±6	37±6	40±6
	Mean	39±5	39±5	38±5	40±5
PND 38	1	38±5	35±4	35±4	35±5
	2	34±4	33±4	33±4	34±7
	3	35±5	33±4	33±5	34±6
	4	36±6	35±5	34±4	34±4
	5	34±4	36±5	34±5	36±6
	Mean	35±3	34±3	34±3	35±4
PND 60	1	37±5	39±3	38±5	37±3
	2	34±2	36±3	35±5	36±5
	3	35±3	36±4	35±4	36±4
	4	36±5	35±4	36±5	36±4
	5	37±5	36±4	37±5	38±5
	Mean	36±3	36±3	36±4	37±3
Females					
PND 22	1	37±7	41±9	37±6	40±8
	2	38±10	38±7	39±6	38±5
	3	36±5	35±7	38±8	40±6
	4	38±8	37±8	41±8	41±6
	5	39±9	38±7	38±5	39±7
	Mean	38±6	38±6	39±5	40±4
PND 38	1	34±4	37±5	38±4	35±4
	2	33±4	37±7	36±4	37±6
	3	33±5	36±5	37±5	37±6
	4	35±5	36±6	35±4	35±5
	5	35±4	35±5	36±5	36±7
	Mean	34±3	36±4	37±3	36±4
PND 60	1	40±5	41±6	40±4	43±5
	2	38±5	40±6	41±5	40±5
	3	37±6	40±6	42±6	41±6
	4	38±8	40±7	42±8	42±5
	5	40±5	42±7	42±7	41±8
	Mean	39±4	41±5	41±4	41±4

[a] Data obtained from Tables 23-24, pages 227-236, MRID 45499501
N = 16/sex/dose

d. Learning and memory testing:

Passive Avoidance: There were no treatment-related effects, and acquisition and retention were appropriate in control animals. The statistical difference observed for low-dose males (Session 1, Latency trial 2) is considered incidental to treatment. Data are summarized in Table 12.

TABLE 12. Passive avoidance performance at PND 24/31(mean ± S.D.) [a]

Test Day/Parameter		Dose (ppm)			
		0	4	40	200
Males					
Session 1 (Learning)	Trials to criterion	3.9±0.9	4.1±1.5	3.7±0.9	3.6±1.0
	Latency trial 1 (sec)	23.2±18.0	25.9±16.6	33.4±39.3	35.8±22.7
	Latency trial 2 (sec)	167.6±25.3	125.2*±54.6	164.0±27.2	162.4±39.1
	Failed to Learn/No. Tested	0/16	0/16	0/16	0/15
Session 2 (Retention)	Trials to criterion	2.4±0.6	3.0±1.3	2.6±1.3	2.5±0.7
	Latency trial 1 (sec)	162.8±38.2	159.8±45.2	143.6±50.9	162.6±37.9
	Latency trial 2 (sec)	174.1±23.6	168.5±36.2	180.0±0.0	166.6±35.4
Females					
Session 1 (Learning)	Trials to criterion	4.2±2.8	3.4±0.6	3.4±0.5	3.4±0.6
	Latency trial 1 (sec)	30.4±29.9	25.4±15.5	23.1±19.0	29.4±33.8
	Latency trial 2 (sec)	140.3±51.2	159.2±41.4	133.4±62.0	165.0±36.4
	Failed to Learn/No. Tested	0/15	0/16	0/16	0/15
Session 2 (Retention)	Trials to criterion	2.5±0.7	2.4±0.5	2.7±0.7	3.1±1.2
	Latency trial 1 (sec)	156.1±44.5	162.4±33.5	151.8±40.1	164.2±32.9
	Latency trial 2 (sec)	164.8±40.7	180.0±0.0	173.6±23.9	155.3±49.2

[a] Data obtained from Table 25, pages 237-239, MRID 45499501. *p<0.05.

Water Maze: There were no treatment-related differences for males or females at any dose level compared to controls with regard to trials-to criterion, time to escape, number of errors, or failure to meet criterion. Data are summarized in Table 13.

TABLE 13. Water maze performance at PND 60/67

Test Day/Parameter		Dose (ppm)			
		0	4	40	200
Males					
Session 1 (Learning)	Trials to criterion	7.9±3.2	7.4±2.1	7.0±1.9	6.4±1.9
	Trial 1 errors (mean ± SD)	1.1±1.4	1.2±1.2	0.7±0.7	0.6±0.9
	Trial 1 duration (sec) (mean ± SD)	22.2±18.2	22.8±17.4	25.1±20.7	15.1±12.2
	Trial 2 errors (mean ± SD)	0.9±1.3	0.7±1.1	0.4±0.6	0.4±0.7
	Trial 2 duration (sec) (mean ± SD)	21.4±19.4	15.5±13.6	13.4±8.4	13.0±14.0
	Failed to meet criterion	2/16 (13%)	0/16 (0%)	0/16 (0%)	0/16 (0%)
Session 2 (retention)	Trials to criterion	5.4±0.8	5.4±0.6	5.8±1.5	6.1±1.7
	Trial 1 errors (mean ± SD)	0.4±0.8	0.5±0.8	0.4±0.9	0.6±1.1
	Trial 1 duration (sec) (mean ± SD)	8.4±4.5	10.2±7.9	9.1±8.0	12.8±15.2
	Trial 2 errors (mean ± SD)	0.2±0.6	0.2±0.8	0.2±0.5	0.2±0.8
	Trial 2 duration (sec) (mean ± SD)	5.3±4.5	4.9±3.6	6.6±7.0	6.9±8.5
Females					
Session 1 (Learning)	Trials to criterion	8.6±3.2	8.4±2.8	8.1±3.1	9.8±3.6
	Trial 1 errors (mean ± SD)	0.9±1.1	0.9±1.0	0.7±1.1	0.9±1.0
	Trial 1 duration (sec) (mean ± SD)	17.8±15.4	19.0±14.8	17.4±14.4	12.3±5.7
	Trial 2 errors (mean ± SD)	1.1±1.2	0.8±0.9	0.4±0.6	0.8±0.8
	Trial 2 duration (sec) (mean ± SD)	14.1±11.2	14.5±8.6	8.4±4.9	13.1±6.1
	Failed to meet criterion	2/16 (13%)	1/16 (6%)	1/16 (6%)	3/16 (19%)
Session 2 (retention)	Trials to criterion	6.1±1.7	6.1±1.7	6.6±2.9	5.9±2.0
	Trial 1 errors (mean ± SD)	0.4±0.9	0.3±0.8	0.1±0.4	0.1±0.3
	Trial 1 duration (sec) (mean ± SD)	7.1±8.7	7.2±9.7	6.6±4.6	5.8±3.9
	Trial 2 errors (mean ± SD)	0.1±0.4	0.1±0.3	0.1±0.5	0.4±1.0
	Trial 2 duration (sec) (mean ± SD)	5.8±4.7	4.3±1.9	4.4±2.6	5.8±5.7

aData obtained from Table 26, pages 240-242, MRID 45499501.

e. **Ophthalmology and Electroretinography:** There were no treatment-related ocular effects in any treated animals compared to controls.

5. **Postmortem results:**

a. **Brain weights:** Mean brain weight data are presented in Table 14. Absolute brain weights of high-dose males and females were significantly ($p \leq 0.05$) decreased compared to controls on PND 11 and 21. Lower absolute brain weight differences were noted in high dose males and females on day 70 but they did not show statistical significance.

TABLE 14. Mean (±SD) Brain Weight Data in Offspring [a]				
Parameter	Dose (ppm)			
	0	4	40	200
Males				
Day 11				
Terminal body weight (g)	21.4±2.9	22.5±2.2	21.2±2.1	15.5*±2.2
Brain weight (g)	1.321±0.087	1.361±0.073	1.339±0.092	1.148*±0.156
Day 21				
Terminal body weight (g)	49.8±3.3	51.6±5.3	48.1±3.9	39.5*±5.0
Brain weight (g)	1.805±0.083	1.770±0.065	1.748±0.062	1.669*±0.107
Termination				
Terminal body weight (g)	311.2±26.3	302.0±23.4	309.2±21.6	275.2*±23.7
Brain weight (g)	1.882±0.059	1.836±0.076	1.869±0.099	1.798±0.085
Females				
Day 11				
Terminal body weight (g)	20.5±2.0	21.5±2.2	20.1±2.7	15.4*±2.1
Brain weight (g)	1.268±0.062	1.310±0.087	1.301±0.082	1.140*±0.138
Day 21				
Terminal body weight (g)	47.0±3.6	49.5±4.2	45.0±4.1	37.6*±4.0
Brain weight (g)	1.713±0.052	1.742±0.045	1.715±0.048	1.608*±0.075
Termination				
Terminal body weight (g)	195.0±18.3	188.9±16.0	192.4±16.2	179.6*±13.5
Brain weight (g)	1.709±0.047	1.734±0.045	1.706±0.077	1.642±0.076

a Data obtained from pages 986-993, MRID 45499501. $p<0.05$.
N = 9-12/sex/dose

b. **Cholinesterase activity:** Results of cholinesterase activity assessment are presented in Table 15. For the PND 11 offspring. The only significant effect noted was a 6% decrease in brain cholinesterase activity. There was a 21-22% decrease in plasma cholinesterase in high-dose male and female offspring on PND 21. RBC and brain cholinesterase activity was not adversely affected in the offspring. Effects in the dams were briefly discussed above (Section II.a.4).

TABLE 15. Cholinesterase activity in dams and offspring

Cholinesterase [mean ± SD (% inhibition relative to control)]	Dose (ppm)			
	0	4	40	200
Lactation day 21 Dams				
Plasma (IU/mL)	0.64 ± 0.11	0.62 ± 0.17 (3)	0.22* ± 0.08 (66)	0.08* ± 0.01 (88)
RBC (IU/mL)	1.14 ± 0.22	0.98 ± 0.19 (14)	0.27* ± 0.17 (76)	0.15* ± 0.13 (87)
Brain (IU/g)	13.1 ± 0.8	12.9 ± 0.7 (2)	10.2* ± 1.9 (22)	3.4* ± 0.6 (74)
Day 11 Male Offspring				
Plasma (IU/mL)	0.79 ± 0.11	0.83 ± 0.04 (+5)	0.83 ± 0.07 (+5)	0.85 ± 0.11 (+8)
RBC (IU/mL)	1.58 ± 0.32	1.62 ± 0.17 (+3)	1.81 ± 0.31 (+15)	1.72 ± 0.30 (+9)
Brain (IU/g)	6.8 ± 0.6	6.9 ± 0.3 (+1)	7.2 ± 0.5 (+6)	7.1 ± 0.8 (+4)
Day 11 Female Offspring				
Plasma (IU/mL)	0.80 ± 0.07	0.79 ± 0.09 (1)	0.78 ±0.07 (3)	0.85 ±0.08 (+6)
RBC (IU/mL)	1.69 ± 0.16	1.59 ± 0.20 (6)	1.73 ± 0.41 (+2)	1.76 ± 0.29 (+4)
Brain (IU/g)	7.0 ± 0.4	7.2 ± 0.3 (+3)	7.2 ± 0.3 (+3)	6.6* ± 0.5 (6)
Day 21 Male Offspring				
Plasma (IU/mL)	0.62 ± 0.06	0.63 ± 0.08 (+2)	0.57 ± 0.10 (8)	0.49* ± 0.16 (21)
RBC (IU/mL)	1.27 ± 0.20	1.45 ± 0.48 (+14)	1.55 ± 0.30 (+22)	1.33 ± 0.58 (+5)
Brain (IU/g)	11.2 ± 0.5	11.5 ± 1.2 (+3)	11.2 ± 0.5 (0)	10.8 ± 0.6 (4)
Day 21 Female Offspring				
Plasma (IU/mL)	0.60 ± 0.09	0.65 ± 0.05 (+8)	0.53 ± 0.12 (12)	0.47* ± 0.19 (22)
RBC (IU/mL)	1.42 ± 0.29	1.39 ± 0.26 (2)	1.47 ± 0.30 (+4)	1.26 ± 0.42 (11)
Brain (IU/g)	11.2 ± 0.5	11.3 ± 0.5 (+3)	11.1 ± 0.6 (1)	10.8 ± 0.7 (4)

Data obtained from Appendix Tables, pages 940-946, MRID 45499501. Percentages calculated by reviewer.

C. Neuropathology

1. **Macroscopic examination:** No treatment-related effects were reported for male or female offspring at postnatal day 21 or study termination.

2. **Microscopic examination:** No significant treatment-related effects were noted on postnatal days 21 or 70.

Brain Morphometry: The anterior to posterior lengths of the cerebrum were marginally decreased ($p<0.05$) for high-dose males and females compared to controls on PND 11. The anterior to posterior length for the cerebellum was also marginally decreased in high-dose males and females, although the effect did not reach statistical significance. No significant morphomeric effects were observed in low- or mid-dose animals on PND 11 or in any treated animals at PND 21 or study termination. Data are summarized in Table 16.

TABLE 16. Mean (±SD) morphometric data in offspring [a]				
Parameter	Dose (ppm)			
	0	4	40	200
Males				
Day 11				
Anterior to posterior cerebrum length (mm)	12.44±0.45	12.56±0.32	12.49±0.40	11.69*±0.73
Anterior to posterior cerebellum length (mm)	7.37±0.43	7.30±0.25	7.46±0.44	7.05±0.45
Day 21				
Anterior to posterior cerebrum length (mm)	14.30±0.37	14.21±0.20	14.24±0.38	13.95±0.47
Anterior to posterior cerebellum length (mm)	8.07±0.42	8.08±0.49	8.02±0.48	8.21±0.29
Termination				
Anterior to posterior cerebrum length (mm)	14.74±0.25	14.66±0.38	14.82±0.39	14.56±0.40
Anterior to posterior cerebellum length (mm)	8.24±0.39	8.07±0.36	8.05±0.46	8.04±0.37
Females				
Day 11				
Anterior to posterior cerebrum length (mm)	12.28±0.29	12.48±0.43	12.42±0.43	11.76*±0.59
Anterior to posterior cerebellum length (mm)	7.36±0 48	7.47±0.52	7.25±0.42	7.09±0.42
Day 21				
Anterior to posterior cerebrum length (mm)	13.97±0.33	14.11±0.23	14.08±0.29	13.81±0.43
Anterior to posterior cerebellum length (mm)	7.87±0.39	8.08±0.30	7.91±0.17	8.14±0.33
Termination				
Anterior to posterior cerebrum length (mm)	14.29±0.29	14.20±0.27	14.10±0.32	14.22±0.25
Anterior to posterior cerebellum length (mm)	7.93±0.40	8.00±0.32	7.64±0.22	7.60±0.30

a Data obtained from pages 986-990, MRID 45499501. p<0.05.
N = 9-12/sex/dose

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the overall NOEL is 4 ppm for dams based on cholinesterase inhibition at 40 and 200 ppm. The investigators also concluded that the overall NOEL for offspring is 40 ppm based on decreased cholinesterase activity, decreased body weight, clinical signs, decreased startle response amplitude (PND 22 only), and a transient decrease in brain weight.

B. **REVIEWER COMMENTS**: No treatment-related effects were seen on survival, food consumption or reproductive parameters. Dams exhibited clinical signs of toxicity, slight tremors, at parturition which , are likely associated with the observed cholinesterase inhibition. Maternal body weight during lactation was decreased at each time point recorded for the high-dose group, with average differences of 8-12% compared to the control.

Cholinesterase activity was decreased ($p<0.05$) in maternal animals at 40 and 200 ppm. Decreases were 66%, 76%, and 22% at 40 ppm, and 88% 87%, and 74% at 200 ppm compared to control for plasma, erythrocyte and brain cholinesterase activities, respectively.

Treatment had no adverse effects on offspring survival. No treatment-related effects on body weight or body weight gains were seen at 4 or 40 ppm pups. At the high dose (200 ppm), pups weighed an average of 16% less than controls on PND 4, 21% on PND 11 and 22% by PND 21. From birth to PND4, high-dose pups gained 37-38% less than controls, and decreased body weight gain continued to be observed for high-dose pups throughout the lactation period. For males, body weights were 21% below controls on day 27, and had partially recovered by the end of the study, to approximately 12% below controls. For high-dose females, body weights were 20% below controls on day 6, and had partially recovered by the end of the study, to approximately 8% below controls. Preputial separation for high-dose males was delayed relative to controls. Additionally, the development of a surface-righting reflex in high dose animals was significantly ($p \leq 0.05$) delayed. The average day of a surface righting was 6.0 days for control and 7.2 days for the high-dose animals.

Motor activity was decreased in males (15%) and females (46%) at the high dose on PND 13. Conversely, motor activity was increased in males (12%) and females (43%) at the high dose on PND 17. Changes occurred in both directions or at a lower magnitude at the low and mid dose groups for both sexes on PND 13 and PND 17. There were no apparent effects on motor activity in males or females on PND 21 and PND 60.

Startle response amplitude for high-dose males and females was decreased compared to controls on day 21, but not on subsequent test days. No treatment-related effects were seen on learning and memory. Absolute brain weights of high-dose males and females were decreased (4-13%) compared to controls on days 11 and 21. The anterior to posterior lengths of the cerebrum were marginally decreased ($p<0.05$) for high-dose males and females compared to controls on PND 11. The anterior to posterior length for the cerebellum was also marginally decreased in high-dose males and females, although the effect did not reach statistical significance

For the day 21 offspring, the only effect was a 21-22% decrease in plasma cholinesterase in high-dose males and female. RBC and brain cholinesterase activity was not adversely affected in the offspring. In accordance with the current policy inhibition of plasma cholinesterase activity alone in the absence of clinical signs is not considered to be appropriate for use as the basis for a LOAEL.

The maternal systemic LOAEL is 200 ppm (16.4 mg/kg/day) based on clinical signs (tremor) and decreased body weight during lactation. The maternal systemic NOAEL is 40 ppm (3.4 mg/kg/day).

The maternal cholinesterase LOAEL 40 ppm (3.4 mg/kg/day) based on decreases in plasma, erythrocyte and brain cholinesterase activity. The maternal cholinesterase NOAEL is 4 ppm (0.4 mg/kg bw/day).

The offspring systemic LOAEL is 200 ppm (16.4 mg/kg/day), based on decreased body weight, body weight gain, delay in preputial separation, delayed surface righting reflex, decreased motor activity, decreased startle response amplitude, decreased absolute brain weights, and changes in the brain morphometrics (cerebrum and cerebellum). The offspring systemic NOAEL is 40 ppm (3.4 mg/kg/day).

The offspring off spring cholinesterase NOAEL is 200 ppm (16.4 mg/kg/day), the highest dose tested. An offspring cholinesterase NOAEL was not established.

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.

C. **STUDY DEFICIENCIES:** Analytical data on homogeneity and stability of the dosing solutions.