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

OFFICE OF PREVENTION PESTICIDES AND TOXIC SUBSTANCES

Date: August 18, 2005

TXR: 0051561

MEMORANDUM

SUBJECT: **DISULFOTON: Data Evaluation Record of a Developmental Neurotoxicity Study**

PC Code: 032501 Reregistration Case #: 0102
DP Barcode: D288336

FROM: Jess Rowland. Chief
Science Information Management Branch
Health Effects Division (7509C)

TO: Christina Sheltema
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Special Review and Reregistration Division (7508C)

THRU: Brenda May, Branch Senior Scientist
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Health Effects Division (7509C)

I. Conclusions

Attached is the Data Evaluation Record for Developmental Neurotoxicity (DNT) Study with Disulfoton (MRID No. 45827601). This study is classified Acceptable and may be used for regulatory purposes. It, however, 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. This classification scheme is applicable only to the Developmental Neurotoxicity studies as determined by DNT Work Group.

II. Action Requested

Review/prepare a Data Evaluation Record for Developmental Neurotoxicity Study with Disulfoton. (MRID No. 45827601).

DATA EVALUATION RECORD

DISULFOTON

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 45827601

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
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Task No. 03-13

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Disclaimer

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

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Date 5/19/05

Template version 11/01

DATA EVALUATION RECORD

TXR#: 0051561

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

PC CODE: 032501

DP BARCODE: D288336
SUBMISSION NO.: S69209

TEST MATERIAL (PURITY): Technical Grade Disulfoton (98.0-98.8%)

SYNONYMS: O,O-Diethyl S-[2-(ethylthio)ethyl] phosphorodithioate; Disyston®

CITATION: Sheets, L. P. (2002) A Developmental neurotoxicity screening study with technical grade Disulfoton (Disyston®) in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 200249; September 20, 2002. MRID 45827601. Unpublished

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

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45827601), Disulfoton (98.0-98.8% a.i., batch # 103-0020) was administered to 30 parent female Wistar rats/dose in the diet at concentrations of 0, 0.5, 2 or 8 ppm from gestation day 0 through postnatal day 21. The average daily intake of Disulfoton was 0, 0.038, 0.156, and 0.670 mg/kg/day during gestation and 0, 0.102, 0.389, and 1.714 mg/kg/day during lactation, for the 0, 0.5, 2.0, and 8 ppm groups, respectively. A Functional Operational Battery (FOB) was performed on 30 dams/dose from gestation days 6, and 20 and on 10 dams/dose on lactation days 11 and 21. On postnatal day 4, litters were reduced in size to yield four males and four females (as closely as possible). Offspring representing at least 20 litters/dose were allocated for detailed clinical observations (abbreviated FOB), assessment of motor activity, assessment of auditory startle response and habituation, assessment of learning and memory, opthalmology, and neuropathology at study termination (day 75 of age). On postnatal day 21 and 70-80, the whole brain was collected from 10 pups/sex/dietary level for micropathologic examination and morphometric analysis. [Morphometric analysis was conducted on brains from rats flushed with sodium nitrite followed

by fixation with 1% gluteraldehyde and 4% paraformaldehyde after administration of anesthetic.] The remaining pups in this set were sacrificed on postnatal day 21 for measurement of cholinesterase activity. Brain, erythrocyte, and plasma cholinesterase activities were measured in offspring (20/dose group) on days 4 and 21 and in dams (10/dose group) on postnatal day 21. Pup physical development was assessed by bodyweight, and sexual maturation of females was assessed by age at vaginal opening. Maturation of males was assessed by age at completion of balano-preputial separation.

In high-dose dams during gestation and lactation, treatment-related effects for maternal animals included repetitive chewing movements and muscle fasciculations which are consistent with signs of cholinergic toxicity and decreased cholinesterase activity. Body weight during lactation was decreased at each time point recorded for the high-dose group, with average differences of 5% on Lactation Day 0 and 8-9% thereafter, compared to controls. Because of the low magnitude of response (i.e.. decreases less than 10%), the lack of dose response, and no decreses during the gestation period, the decreases seen at the high dose only during lactation were not considered to be adverse or of toxicological significance.

For maternal systemic toxicity, the LOAEL is 8 ppm (0.670 mg/kg/day) based on cholinergic signs. The maternal systemic NOAEL is 2 ppm (0.156 mg/kg/day) in the diet.

Cholinesterase activity was decreased ($p<0.05$) in maternal animals at 0.5, 2, and 8 ppm. Decreases were 10%, 27%, and 10% at 0.5 ppm, 61%, 73%, and 65% at 2 ppm, and 86%, 86%, and 86% at 8 ppm compared to control values for plasma, erythrocyte and brain cholinesterase activities, respectively.

For maternal cholinesterase inhibition, the LOAEL is 0.5 ppm (0.038 mg/kg/day) based on statistically significant decreases in erythrocyte and brain cholinesterase activities. The maternal cholinesterase inhibition NOAEL was not determined.

In offspring, male and female body weights were significantly decreased at 8 ppm on lactation days 4, 11, 17 and 21. By postnatal day (PND) 28 post weaning, male pup weight was significantly decreased at 2.0 and 8.0 ppm and remained so until postnatal day 70. During this period female postnatal pup weight was decreased only at the highest dose tested (8.0 ppm). Preputial separation in males was unaffected by treatment. Although the onset of preputial separation was delayed an average of 1.1 days in high-dose animals, the effect is considered toxicologically insignificant as it falls within the historical control range. The mean age for attainment of vaginal opening for females was delayed in mid- and high-dose animals

No effects were seen in FOB, motor/locomotor, auditory startle/latency, learning and memory/latency tests or ophthamology. Habituation was noted in motor/locomotor and auditory startle tests. Absolute brain weights were unchanged. Perfused 21 day old brains showed no difference from control values and the groups showed no difference from controls in absolute brain weight. Morphometric data on perfused brains showed no definitive effects. The report indicated that the forebrain frontal and parietal cortex were statistically significantly increased in postnatal day 70-80 males and that the forebrain caudate putamen in postnatal males and females

were statistically significantly increased at 8.0 ppm. Since the report author did not believe the measurements were treatment related, the corresponding mid and low dose values were not reported. The author did not believe that the values were treatment related because of the low magnitude in response and lack of corresponding microscopic findings.

For offspring systemic toxicity, the LOAEL is 2 ppm (0.156 mg/kg/day) based on delayed vaginal opening in females. The offspring systemic toxicity NOAEL is 0.5 ppm (0.038 mg/kg/day).

Statistically significant decreases in cholinesterase activity were seen in the day 4 d male and female (combined) pups as well as in the Day 21 male and female pups On PND day 4, offspring showed statistically significant decreases in plasma (11%) and erythrocyte (50%) cholinesterase inhibition at 8 ppm. On PND 21, male/female pups showed cholinesterase inhibition in plasma (43%/41%), erythrocyte (53%/56%) and brain (30%/30%) only at 8.0 ppm.
There was a non-dose related but statistically significant decrease in brain cholinesterase activity at 2 ppm only. This decrease was attributed to a low value in one pooled sample and is considered incidental to treatment.

For offspring cholinesterase inhibition, the LOAEL is 8 ppm (0.670 mg/kg/day) based on decreased plasma and erythrocyte cholinesterase activity on PND 4 (male and female pups combined) and decreased cholinesterase activity in all three compartments in male and female pups on PND 21. The offspring cholinesterase inhibition NOAEL is 2 ppm (0.156 mg/kg/day).

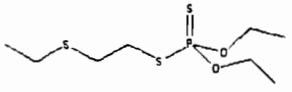
This study is classified **Acceptable** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control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

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Disulfoton

Description:	Light yellow, oily liquid
Lot/Batch #:	103-0020
Purity:	98.0-98.8 % a.i.
Compound Stability:	Confirmed for 6 months
CAS # of TGAI:	298-04-4
Structure:	

2. **Vehicle and/or positive control:** acetone solvent in the diet

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

Species:	Rat
Strain:	Wistar Hannover Crl:WI(Glx/BRL/Han) IGS BR
Age at study initiation:	females: at least 12 wks; males: at least 15 weeks (breeders only)
Wt. at study initiation:	197.3-263.4 g
Source:	Charles River Laboratories
Housing:	Individually or with litter in stainless steel grid or plastic cages
Diet:	Purina Mills Rodent Lab Chow 5002, ad libitum
Water:	Tap water, ad libitum
Environmental conditions:	**Temperature:** 19-25°C **Humidity:** 30-70% **Air changes:** 10-15/hour **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least 6 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: April 29, 2001; End: August 3, 2001

2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals (30/dose group) from gestation day 0 through lactation day 21 (approximately 30/group GD 0-21, approximately 20/group LD 0-21 and abreviated FOB with approximately 10/group, LD 11 and 21). Pups were weaned onto control diets on postnatal day 21, after which time maternal animals were killed. F_1 pups remained on study up to postnatal days 70-80 (approximately 39/group to 63/group).

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.

Sixteen pups/sex/group 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, animals were sacrificed by perfusion and brain weights recorded.

Brain, erythrocyte, and plasma cholinesterase activities were measured in offspring on postnatal days 4 (culled offspring) and 21 (Set D) and in dams on lactation day 21. On postnatal day 4, approximately 20 pups/dietary level that were randomly selected for culling; samples from male and female pups within a litter were pooled to provide adequate samples for blood measures. On day 21, samples were collected from 10 dams/dose group and 5-10 pups/dose group from set D.

TABLE 1. Study design

Experimental parameter		Dose (ppm in diet)			
		0	0.5	2	8
Maternal animals					
		Aprox. No. of maternal animals assigned			
Clinical observations (GD 6,20)		30	30	30	30
Elective sacrifice, presumably because inadequate numbers of males or females (GD 20 to LD 24)[a]		8	7	9	11
Clinical observations (LD 0-21)		22	23	21	19
FOB (LD 11, 21)		10	10	10	10
Erythrocyte, Plasma, and Brain Cholinesterase Activity (LD 21)		10	10	10	10
Offspring					
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) Brain Weight (PND 70-80)	10/sex 10/sex	10/sex 10/sex	10/sex 10/sex	10/sex 10/sex
Set D	Gross necropsy and brain measurements (PND 21)*	10/sex	10/sex	10/sex	10/sex
	Erythrocyte, plasma, and brain cholinesterase activity (PND 21)	5-10/ sex	5-10/ sex	5-10/ sex	5-10/ sex
culled	Erythrocyte, plasma, and brain cholinesterase activity (PND 4)**	20	20	20	20

*Page 23 of MRID 45827601 states that approval was granted by OPPTS/OPP/HED staff to replace PND 11 with PND 21 for neuropathology for this study. [a] Elective sacrifice: In some cases liters were born dead, died before lactational day 4 or had inadequate numbers of males or females to reduce the litter to 4/sex/litter.

**Samples from male and female pups within a litter were pooled to provide adequate samples for blood measures.

5. **Dose selection rationale:** Dose levels were chosen based on the results from two-generation reproduction studies in Sprague-Dawley rats. In the first study (Bayer Report 90965; MRID 00157511), Disulfoton was administered in the diet at levels of 0, 1, 3, and 9 ppm beginning 15 weeks before mating. No signs of toxicity were noted during the premating phase; however, 9 ppm parental females exhibited tremors during the mating phase. Reproductive and litter indices were adversely affected at 9 ppm. Decreased brain cholinesterase activity was noted in F_1 offspring sacrificed at weaning at 3 ppm (males 24% and females 32% decrease) and 9 ppm (males 50% and females 59% decrease).

   In the second study (Bayer Report 108002; MRID 44440801), Disulfoton was administered in the diet at levels of 0, 0.5, 2, and 9 ppm beginning 10 weeks before mating. At 2 and 9 ppm, parental females had decreased plasma (-52% at 2 ppm, -88% at 9 ppm), erythrocyte (-80% at 2 ppm, -90% at 9 ppm), and brain (-59% at 2 ppm, -86% at 9 ppm) cholinesterase activities. At 9 ppm, parental females had tremors and decreased activity, resulting in one death. During lactation, F_1 offspring from the 9 ppm group were weak, cold and/or without milk in their stomachs, and had increased mortality. These effects suggest poor maternal care or insufficient milk production. Reproductive parameters were adversely affected only in the second generation. High-dose F_1 males and females exhibited decreased ($p \leq 0.05$) plasma, erythrocyte and brain cholinesterase activities at postnatal days 4 and 21.

   Based on the results of these two studies, the doses selected for the developmental neurotoxicity study were 0, 0.5, 2, and 8 ppm. The 8 ppm level was selected to produce evidence of toxicity and approximate a MTD. The 0.5 ppm dose was selected to produce no signs of toxicity and no cholinesterase inhibition, and the 2 ppm level was selected as an intermediate dose to assist in establishing compound-related effects.

6. **Dosage administration:** Disulfoton was administered to parent female Wistar rats in the diet at levels of 0, 0.5, 2 or 8 ppm from gestation day 0 through postnatal day 21. The test substance intake was 0, 0.038, 0.156, and 0.670 mg/kg/day, respectively, for analytically-determined concentrations of 0, 0.5, 2.0, and 7.9 ppm in the diet during gestation. The test substance intake was 0, 0.102, 0.389, and 1.714 mg/kg/day, respectively, for analytically-determined concentrations of 0, 0.5, 2.0, and 7.9 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. Acetone was used as the solvent to dissolve the test article for mixing in the diet and was allowed to evaporate before the feed was given to the animals. The control diet was similarly prepared, excluding the test substance. Concentrations of the test substance in the diet were measured by gas chromatography four times (weeks 1, 2, 3, and 6) during the in-life phase of the study. Homogeneity and stability data from a previous study (utilizing concentrations of 0.5 and 9 ppm) were presented.

Results:

Homogeneity analysis: was not performed for this study; however the study report states, "At nominal concentrations of 0.5 ppm and 9.0 ppm, Disulfoton is homogeneous and stable in the diet for at least 4 days at room temperature and 28 days at freezer conditions."

Stability analysis: was not performed for this study; however the study report states, "At nominal concentrations of 0.5 ppm and 9.0 ppm, disulfoton is homogeneous and stable in the diet for at least 4 days at room temperature and 28 days at freezer conditions."

Concentration analysis: The 0.5, 2, and 8 ppm dietary levels averaged 98.4%, 98.5%, and 98.2% of the nominal concentration, respectively.

The analytical data indicated the concentration of disulfoton 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 (30/group) from gestational day 0 to 21 and lactational day 0 to 21 (about 20/group) that remained on study.

b. **Maternal animals (abreviated FOB)**:
Thirty dams per group were observed (by observers blind to the treatment group) outside the home cage during the gestation dosing period (days 6 and 20), and a minimum of 10 rats/dietary level that were maintained on study were observed at lactational day 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 data were recorded weekly for gestation days 0, 6, 13, and 20, and lactation days 0, 7, 14, and 21. Individual maternal food consumption was recorded two times per week for gestation days 0-4, 4-6, 6-10, 10-13, 13-17, and 17-21 and lactation days 0-4, 4-7, 7-11, 11-14, 14-18, and 18-21.

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 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 approximately118 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 22 and 29, 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 acquistion 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 M-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 M-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-free 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:**

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

10. **Postmortem observations:**

Cholinesterase measurements: Blood and brain samples were collected from 10 dams/dose group on lactation day 21 and from 5-10 pup/sex/dose group on postnatal day 21. Blood and brain samples were also collected from 20 pups/dose group (selected for culling) on postnatal day 4, and blood samples from male and female offspring within a litter were pooled to provide adequate samples for blood measurements. Blood samples were collected from the orbital plexus of dams and offspring on postnatal day 21 and by decapitation of offspring on postnatal day 4 for determination of plasma and erythrocyte cholinesterase activities. Brain tissues were collected immediately after blood collection for the determination of brain cholinesterase activity.

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 21 or 70-80. These animals were subjected to postmortem examinations as described below.

At postnatal day 21, up to 10 pups/sex/group were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg) and perfused via the left ventricle with a sodium nitrite flush followed by fixation with 1% gluteraldehyde and 4% paraformaldehyde. The brain was collected, weighed, and post-fixed with 10% buffered formalin. 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 and luxol fast blue/cresyl violet. 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 were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg) and perfused via the left ventricle with a sodium nitrite flush followed by fixation with 1% gluteraldehyde and 4% 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. 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 system 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) ▸× 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:

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

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

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

3. **Positive and historical control data:** Positive controls studies (MRID# 45441302, 45441303, 4564601, and 4564602 are under review.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and Clinical Observations:** One high-dose dam was found partially paralyzed on lactation day 18 and was found dead later that day. Necropsy revealed hemorrhage in the spinal cord was responsible for the paralysis, thus, it is likely that this death was incidental to treatment. There were no other maternal deaths before scheduled termination.

   Treatment-related clinical signs were observed during gestation only in high-dose dams and included repetitive jaw chewing (total incidence: 0/30 control, 0/30 low-dose, 0/30 mid-dose, 11/30 high-dose), muscle fasciculation (total incidence: 0/30 control, 0/30 low-dose, 0/30 mid-dose, 6/30 high-dose), and jerking movements (total incidence: 0/30 control, 0/30 low-dose, 0/30 mid-dose, 3/30 high-dose). Effects similar to those observed during gestation were seen during lactation in the dams that remained on the study, but only at the high dose (8 ppm). Although no statistical analyses were conducted on these parameters, it is obvious that these effects, consistent with cholinergic toxicity from carbamate insecticides, were mostly noted

during week 3 of gestation and lactation when exposure was greatest due to high food consumption. There were no other treatment-related clinical signs observed during gestation or lactation (Table 2).

Table 2: Clinical observations of dams during gestation and lactation. Data obtained form page 63-64 and 70-71 of the report.

Dose level (ppm)	Control	0.5	2	8
Elective sacrifice about GD 20 to LD 0-4[a]	8	7	9	11
Repetative chewing movements				
GD 6	0/30	0/30	0/30	0/30
GD 20	0/30	0/30	0/30	1/30
Total during gestation GD 0-20, LD 0-4	0/30	0/30	0/30	11/30
LD 11	0/22	0/23	0/21	4/20
LD 21	0/22	0/23	0/21	9/19
Total during lactation, LD 0-21	0/22	0/23	0/21	16/19
Muscle fasciculations				
GD 6	0/30	0/30	0/30	0/30
GD 20	0/30	0/30	0/30	1/30
Total during gestation, GD 0-20, LD0-4	0/30	0/30	0/30	6/30
LD 11	0/22	0/23	0/21	0/20
LD 21	0/22	0/23	0/21	0/19
Total during lactation, LD 0-21	0/22	0/23	0/21	2/19
Jerking movements				
GD 6	0/30	0/30	0/30	0/30
GD 20	0/30	0/30	0/30	1/30
Total during gestation. GD 0-20, LD0-4	0/30	0/30	0/30	3/30
LD 11	0/22	0/23	0/21	1/20
LD 21	0/22	0/23	0/21	1/19
Total during lactation, LD 0-21	0/22	0/23	0/21	9/19

[a] Elective sacrifice of dams because the litter was unacceptable, presumably because of dead pups inadequate numbers of males or females by lactational day 4 [Data obtained page 269-277 and 289-292]. GD = Gestational day; LD= Lactational day. Numbers presented are animals effect was seen/total animals observed.

2. Abbreviated FOB in Maternal Animals:

Treatment-related effects were seen in the abbreviated FOB during gestation and lactation in high dose dams (Table 2). On GD 20, these effects include repetitive chewing and muscle fasciculation. An increased incidence of these effects were also seen on lactation day 11. These effects were attributed to cholinergic toxicity, similar to the clinical signs described above.

TABLE 2. Incidence of treatment-related FOB effects in dams

	Dose (ppm)			
	0	0.5	2	8
Repetitive chewing movements				
Gestation day 6	0/30	0/30	0/30	0/30
Gestation day 20	0/30	0/30	0/30	2/28
Lactation day 11	0/10	0/10	0/10	5/10*
Lactation day 21	0/10	0/9	0/10	1/8
Muscle fasciculations				
Gestation day 6	0/30	0/30	0/30	0/30
Gestation day 20	0/30	0/30	0/30	4/26*
Lactation day 11	0/10	0/10	0/10	1/10
Lactation day 21	0/10	0/9	0/10	0/9
No Jerking movements were seen in this phase of the study				

Data obtained from Table 17, pages 110, 116, 122, & 128. MRID 45827601. *p≤ 0.05.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 3. 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 5% on lactation day 0 and 8-9% thereafter, compared to controls. Decreases of 4-5% ($p \leq 0.05$) were also observed for low-dose animals on lactation day 4 and for mid-dose animals on lactation day 14, but these changes are considered incidental to treatment.

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

TABLE 3. Selected mean (±SD) maternal body weight and food consumption[a]

Observations/study interval	Dose (ppm)			
	0	0.5	2	8
Gestation (n= 25-30)				
Body wt. Gestation day 0 (g)	229.8±2.12	224.2±2.15	225.3±2.44	228.0±2.72
Body wt. Gestation day 6 (g)	247.0±3.85	236.2±4.14	243.5±3.23	244.4±3.09
Body wt. Gestation day 13 (g)	274.9±2.91	265.8±3.77	269.2±2.81	268.7±2.91
Body wt. Gestation day 20 (g)	335.4±4.43	328.8±4.17	322.1±6.24	326.5±5.39
Wt. gain gestation days 0-20 (g)	105.5±3.48	104.6±2.90	96.8±5.27	98.5±4.48
Food consumption gestation days 0-4 (g/day)	18.3±0.74	19.1±1.11	17.6±0.67	18.0±0.65

TABLE 3. Selected mean (±SD) maternal body weight and food consumption[a]				
Observations/study interval	Dose (ppm)			
	0	0.5	2	8
Food consumption gestation days 10-13 (g/day)	20.6±0.43	18.0±0.78*	20.1±0.53	22.0±0.51
Food consumption gestation days 17-20 (g/day)	21.2±0.64	19.5±0.83	20.8±0.69	21.9±1.03
Lactation (n=19-30)				
Body wt. lactation day 0(g)	262.3±3.06	255.0±2.80	252.4±3.78	248.8±3.63* (5%)
Body wt. lactation day 4 (g)	281.1±3.30	266.1±4.15*	269.1±3.78	258.8±4.54** (7.9%)
Body wt. lactation day 7 (g)	288.3±2.72	277.6±4.59	278.5±3.39	264.2±4.24** (8.4%)
Body wt. lactation day 14(g)	306.4±2.19	293.5±6.16	293.5±3.88*	277.5±3.64** (9.4%)
Body wt. lactation day 21(g)	298.2±3.28	289.2±3.88	288.7±2.99	273.1±3.66** (8.4%)
Food consumption lactation days 0-4 (g/day)	38.9±4.19	40.2±4.77	38.5±4.85	58.3±10.08
Food consumption lactation days 11-14 (g/day)	60.8±1.17	59.2±1.73	59.0±1.10	56.8±3.22
Food consumption lactation days 18-21 (g/day)	76.2±2.86	75.8±1.84	74.7±2.33	72.2±3.00

[a]Data obtained from Tables 3 & 4 pages 65-68 and Tables 6 & 7 pages 73-76, MRID 45827601. *p≤ 0.05. **p≤ 0.01. Number in parentheses is % difference 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 4.

TABLE 4. Reproductive Performance [a]				
Observation	Dose (ppm)			
	0	0.5	2	8
Number Mated	30	30	30	30
Mating Index (%)	100.0	100.0	100.0	100.0
Fertility Index (%)	93.3	100.0	90.0	90.0
Gestation index (%)	100.0	100.0	100.0	100.0

[a]Data obtained from Table 1, pages 60-61. MRID 45827601.

4. **Maternal postmortem results:** Results for the cholinesterase studies in dams are presented in Table 16 below, along with the results for the offspring. For dams, a dose-related decrease in cholinesterase activity was noted on lactation day 21 at all dose levels. Percent inhibition (*p≤0.05) compared to controls was as follows:

Plasma: 10% at 0.5 ppm, 61%* at 2 ppm, and 86%* at 8 ppm
Erythrocyte: 27%* at 0.5 ppm, 73%* at 2 ppm, and 86%* at 8 ppm
Brain: 10%* at 0.5 ppm, 65%* at 2 ppm, and 86%* at 8 ppm

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 5. 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 5. Litter size and viability [a]

Observation	Dose (ppm)			
	0	0.5	2	8
Number of Litters	22	23	21	19
Total number born	255	264	251	227
Number born live	254	263	250	227
Number born dead	1	1	1	0
Mean No. of viable pups:				
Day 0	12	11	12	12
Day 4 [b]	11	11	12	12
Day 4 [c]	8	8	8	8
Day 21	8	8	8	8
Live birth index (%)	99.5	99.7	99.5	100
Viability index	98.5	98.6	98.9	98.3
Lactation index	100	98.9	100	100

[a]Data obtained from Table 9, pages 80-82, MRID 45827601.
[b]Before standardization (litter size reduction). [c]After standardization (litter size reduction).

2. **Body weight:** Body weights were comparable at birth across all dose groups; however, by PND 4, high-dose pups weighed an average of 10% less than controls. By day 21, high-dose pups weighed 15-16% less than controls. No body weight effects were noted at 0.5 or 2 ppm. Body weight gain was also decreased in high-dose pups. From birth to PND 21, high-dose pups gained 18% less than controls. 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 6.

TABLE 6. Mean (±SD) pre-weaning pup body weights and body weight Gain (g) [a]

Postnatal Day	Dose (ppm)							
	0	0.5	2	8	0	0.5	2	8
	Males				Females			
0	6.0±0.11	6.0±0.10	5.8±0.09	5.8±0.09	5.8±0.11	5.7±0.09	5.5±0.09	5.6±0.10
4[b]	10.1±0.24	9.8±0.26	9.8±0.21	9.1±0.30 (9.9%)[d]	9.8±0.24	9.5±0.26	9.3±0.23	8.8±0.33 (10%)[d]
4[c]	10.1±0.24	9.7±0.25	9.9±0.21	9.1*±0.31 (9.9%)[d]	9.8±0.24	9.5±0.26	9.3±0.24	8.8±0.32 (10%)[d]
11	26.0±0.62	24.9±0.65	25.5±0.52	21.5**±0.65 (17%)[d]	25.3±0.65	24.4±0.64	24.4±0.54	21.0**±0.75 (17%)[d]
17	40.4±0.75	38.5±0.81	39.3±0.74	32.9**±0.71 (19%)[d]	39.3±0.74	37.6±0.81	37.7±0.71	32.2**±0.81 (18%[d])
21	51.3±0.96	49.3±0.91	49.2±0.85	43.0**±1.01 (16%)[d]	49.7±0.93	48.0±0.90	47.0±0.82	42.0**±1.05 (15%)[d]
Weight gain Days 0-4	4.1±0.15	3.8±0.20	4.0±0.15	3.3*±0.25 (20%)[d]	4.0±0.15	3.8±0.19	3.9±0.16	3.2*±0.25 (20%)[d]
Weight gain Days 4-11	15.9±0.42	15.1±0.46	15.6±0.34	12.4**±0.41 (22%)[d]	15.5±0.45	15.0±0.44	15.1±0.34	12.2**±0.50 (21%)[c]
Weight gain Days 4-17	30.3±0.57	28.8±0.70	29.4±0.62	23.8**±0.50 (21%)[d]	29.5±0.57	28.1±0.69	28.3±0.58	23.4**±0.58 (21%)[c]
Weight gain Days 4-21	41.2±0.77	39.6±0.77	39.4±0.70	33.9**±0.77 (18%)[d]	39.9±0.75	38.6±0.74	37.7±0.65	33.2**±0.80 (17%)[c]

[a] Data obtained from Tables12-13, pages 91-96. MRID 45827601. *p<0.05. **p<0.01.
[b] Before standardization (litter size reduction).
[c] After standardization (litter size reduction).
[d] (%) decrease compared to controls, calculated by reviewer

Body weights were decreased in high-dose males and females compared to controls following weaning (Table 7). Body weights for high-dose males and females were 10-13% less on day 28 and had partially recovered by the end of the study, to approximately 6-8% below controls. For mid-dose males, body weights were 3-5% below controls throughout the post-weaning period. No biologically-significant postweaning body weight effects were noted in mid- and low-dose females or in low-dose males. Although post weaning body weights were dose related [PND 28 - 70 (4.3% - 4.5%)] in males after secession of dosing and were statistically significantly decreased at 2 ppm compared with control, it was determined that these changes were incidental and not adverse or toxicologically significant due to the low magnitude in the response (i.e., less than 10% decrease) and no further body weight decrease was noted.

TABLE 7. Mean (±SD) post-weaning pup body weights (g) [a]								
Postnatal day	Dose (ppm)							
	0	0.5	2	8	0	0.5	2	8
	Males				Females			
28	83.3±7.3	80.7±8.0	79.7*±7.1 (4.3%)	72.4*±7.9 (13%)	81.4±7.0	79.3±7.1	79.1±7.7	73.1*±7.1 (10%)
35	132.4±12.0	126.4*±19.0 (4.5%)	127.0±13.0 (4.0%)	116.9*±11.7 (12%)	116.7±9.0	115.3±9.9	114.6±8.5	108.7*±9.2 (6.9%)
42	178.7±12.1	173.0±18.0	171.9*±14.1 (3.8%)	160.5*±13.9 (10%)	141.3±10.9	137.3±11.6	137.2±9.4	130.9*±9.9 (7.4%)
49	220.4±15.9	214.1±21.8	212.2*±16.5 (3.7%)	198.8*±17.4 (9.8%)	158.9±11.7	154.7±13.4	155.3±11.0	148.1*±10.8 (6.8%)
56	264.6±20.2	258.0±25.1	255.1*±20.1 (3.6%)	240.6*±21.6 (9.0%)	174.7±13.7	172.5±15.5	172.7±12.8	167.5*±13.0 (4.1%)
63	299.3±22.9	292.2±28.1	285.6*±21.4 (4.6%)	272.4*±23.4 (8.9%)	184.1±14.9	180.3±17.2	181.6±14.7	174.9*±13.2 (5.0%)
70	328.2±26.2	320.5±29.9	313.3*±24.0 (4.5%)	300.6*±26.0 (8.4%)	199.0±17.2	193.7±18.4	195.5±15.9	187.2*±14.4 (5.9%)

[a] Data obtained from Table15, pages 101-103, MRID 45827601. *p<0.05. Body weights were determined on 39 to 63 rats/group. Number in parentheses = % decrease compared to controls, calculated by reviewer

Food consumption (untreated feed) was decreased ($p<0.05$) in treated males compared to controls; however, the decreases are considered incidental to treatment due to the small magnitude and lack of a clear dose-response relationship. For high-dose males, food consumption averaged 5-7% less than controls only on days 56, 63, and 70. For mid-dose males, food consumption averaged 4-6% less than controls only on days 49, 56, 63, and 70, and low-dose males had 4-5% lower food consumption on days 63 and 70. No postweaning food consumption differences were noted in females.

Food Efficiency: There was no dose related effects on food efficiency (Table 8).

Table 8: Food efficiency calculations PND 28 to 60 rats (MRID# 45827601). Data from page 102 & 105 of the report.							
Postnatal male body weight							
Dose/Postnatal day	28	35	42	49	56	63	70
0 ppm	83.3	132.4	178.7	220.4	264.6	299.3	328.2
0.5 ppm	80.7	126.4	173.0	214.1	258.0	292.2	320.5
2 ppm	79.7*	127.0	172.9*	212.2*	255.1*	285.6*	313.3*
8 ppm	72.4*	116.9*	160.5*	198.8*	240.6*	272.4*	300.6*
Postnatal male body weight gain							
0 ppm		49.1	46.3	41.7	44.2	34.7	28.9
0.5 ppm		45.7	46.6	41.1	43.9	34.2	28.3
2 ppm		47.3	44.9	40.3	42.9	30.5	27.7
8 ppm		45.5	43.6	38.3	41.8	31.8	28.2

Postnatal male food consumption							
0 ppm		18.63	21.1	23.2	25.2	26.5	26.3
0.5 ppm		19.9	20.8	22.8	24.3	25.5*	25.0*
2 ppm		17.8	20.7	22.5	23.9*	24.5*	24.5*
8 ppm		20.1	21.0	22.3*	23.9*	24.8*	25.1*
	Mean	Postnatal male food efficiency					
0 ppm	1.80	2.64	2.19	1.80	1.75	1.31	1.10
0.5 ppm	1.80	2.50	2.24	1.80	1.81	1.34	1.13
2 ppm	1.80	2.66	2.17	1.79	1.80	1.24	1.13
8 ppm	1.70	2.26	2.08	1.72	1.75	1.28	1.12

* Statistically significant at $p<0.05$.

3. Developmental landmarks:

a. Sexual maturation: Preputial separation in males was unaffected by treatment. Although the onset of preputial separation was delayed an average of 1.1 days in high-dose animals, the effect is considered toxicologically insignificant as it falls within the historical control range. The mean age for attainment of vaginal opening for females was delayed in mid- and high-dose animals. The data are presented in Table 9.

TABLE 9. Mean (±SD) age of sexual maturation (days) [a]

Parameter	Dose (ppm)			
	0	0.5	2	8
N (M/F)	64/66	67/68	62/63	57/57
Preputial separation (males)	44.0±0.21	44.3±0.24	44.5±0.26	45.1*±0.33
Vaginal opening (females)	33.5±0.28	34.4±0.35	34.9*±0.40	35.5*±0.58

[a] Data obtained from Table 14, pages 99-100, MRID 45827601. *$p<0.05$. Historical control range was 45.6 to 45.4 days for the day of preputial separation. Historical controls ranged from day 33.1 to 34.6 days for the day of vaginal opening.

b. Pupil constriction: No treatment-related effects were noted. All control and treated rats exhibited pupil constriction on PND 21.

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). The number of pools of urine may slightly increased only in females at 8 ppm on postnatal day 60.

b. Motor/locomotor activity: Total activity data are presented in Tables 10 and 11. No treatment-related overall or interval motor or locomotor activity effects were noted. For motor activity in control males and females, habituation was apparent on all four test days, but it was pronounced in males and females postnatal day 17, 21 and 60. For locomotor

activity, habituation was achieved on all test days except PND 13, when activity was low due to the developmental stage of the rats. Habituation was not as pronounced from the first session to the last session on the remaining test days as noted in motor activity (data present in report, but not in this review).

TABLE 10. Mean (±S.D.) motor activity data (total activity counts for session) [a]

Test day	Dose (ppm)			
	0	0.5	2	8
Males				
PND 13	65±62	118±86	98±97	62±48
PND 17	257±128	269±119	313±153	276±158
PND 21	282±117	329±113	391±171	308±91
PND 60	434±113	548±109	547±146	525±111
Females				
PND 13	72±58	70±61	86±61	103±82
PND 17	245±129	227±122	310±135	280±186
PND 21	334±137	293±121 41	320±103 32	329±116
PND 60	742±149	693±167 24	761±229 30	807±159

[a] Data obtained from Table 19, pages 189-191, MRID 45827601.
N = 15-16/sex/dose.

TABLE 11. Mean (±S.D.) locomotor activity data (total activity counts for session) [a]

Test Day	Dose (ppm)			
	0	0.5	2	8
Males				
PND 13	11±17	15±13	17±28	7±9
PND 17	79±53	73±39	82±45	79±56
PND 21	85±38	97±33	117±51	94±32
PND 60	286±112	370±101	383±120	341±103
Females				
PND 13	6±12	14±18	11±10	19±28
PND 17	71±48	66±41	97±52	92±64
PND 21	97±41	96±48	102±36	98±36
PND 60	482±129	438±147	496±168	515±101

[a] Data obtained from Table 20, pages 192-194, MRID 45827601.
N = 15-16/sex/dose.

c. **Auditory startle reflex :** Peak amplitude data are summarized in Table 12 and latency data are summarized in Table 13. Statistically significant decreases in mean auditory startle peak amplitude are observed at various times and doses in all the female groups. Analyses of the individual data for all blocks for PND 22, PND 38 and PND 60 suggest a treatment-related effect between treated and control groups; but no dose response (see Attachment 1). In PND 38 and PND 60 offspring (but not in PND 22), the magnitude of the decrease seen at the low dose is similar to that seen at the high dose group and the mid dose group response is not as

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strong as either the low or the high groups (this may be due to a single pup that recorded the highest startle response for the mid-dose group at each block). Although changes in auditory startle were seen at a lower dose than that which caused delayed vaginal opening, these changes were not used as the basis for the LOAEL because: 1) there was high variability in the control group, 2) most treated values fell within the control range, and 3) there was a lack of an effect at the mid dose.

TABLE 12. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]

	Trial block	Dose (ppm)			
		0	0.5	2	8
Males					
PND 22	1	54±22	45±17	50±22	52±20
	2	53±20	49±22	47±17	54±19
	3	49±26	45±26	43±19	48±22
	4	41±23	43±26	44±22	44±18
	5	38±25	35±19	39±20	37±22
	Mean	47±22	43±21	44±18	47±18
PND 38	1	140±85	128±75	108±53	126±81
	2	134±101	120±92	92±54	108±65
	3	95±87	97±75	88±42	104±63
	4	81±71	75±63	73±41	81±45
	5	68±39	70±53	60±34	78±52
	Mean	104±66	98±68	84±40	99±57
PND 60	1	383±167	297±163	271±189	309±199
	2	278±160	270±157	197±146	259±164
	3	197±123	189±136	168±97	197±124
	4	179±94	146±88	156±96	180±109
	5	122±46	136±78	112±48	166±96
	Mean	232±100	208±108	181±102	222±127
Females					
PND 22	1	57±22	42±15	51±17	49±17
	2	62±30	40±18	52±25	51±21
	3	65±34	39±19*	48±20	49±21
	4	53±25	34±17*	43±17	41±16
	5	47±26	30±15	38±15	37±17
	Mean	57±26	37±15*	46±17	45±17
PND 38	1	135±82	72±34	120±124	73±41
	2	125±86	71±43*	105±55	69±41*
	3	98±56	63±49	89±65	59±27
	4	85±57	49±28	65±51	43±25*
	5	69±50	45±29	62±39	41±22
	Mean	102±62	60±31*	88±62	57±24*

TABLE 12. Auditory startle reflex peak amplitude data (mean g ±S.D.) [a]					
	Trial block	Dose (ppm)			
		0	0.5	2	8
PND 60	1	219±164	118±88*	175±79	107±45*
	2	233±179	125±104*	194±87	131±98
	3	178±161	95±70	130±48	98±54
	4	129±130	84±58	114±52	73±34
	5	115±109	61±34	102±73	66±30
	Mean	175±142	96±65*	143±54	95±45*

[a]Data obtained from Tables 22-23, pages 217-222, MRID 45827601. *p<0.05 N = 15-16/sex/dose

TABLE 13. Auditory startle latency to peak data (mean msec ±S.D.) [a]					
	Trial block	Dose (ppm)			
		0	0.5	2	8
Males					
PND 22	1	38±7	37±8	38±7	39±9
	2	36±6	35±8	38±7	33±6
	3	36±5	33±4	37±9	35±9
	4	37±7	33±5	36±7	38±11
	5	38±8	35±6	37±8	36±7
	Mean	37±4	35±5	37±6	36±7
PND 38	1	34±3	34±4	33±5	34±6
	2	33±3	33±6	32±5	32±4
	3	36±5	33±6	31±3	32±3
	4	35±5	37±9	34±6	34±5
	5	35±5	35±6	33±6	35±5
	Mean	34±4	34±5	33±4	33±3
PND 60	1	38±2	38±5	38±2	37±3
	2	37±3	36±4	36±3	36±4
	3	36±3	36±4	34±3	34±4
	4	35±2	35±5	35±4	35±5
	5	37±3	37±6	36±5	35±3
	Mean	36±2	36±4	36±2	36±3
Females					
PND 22	1	39±10	45±9	40±7	40±9
	2	36±8	38±8	38±10	58±10
	3	37±10	37±6	35±8	36±11
	4	35±7	38±6	34±8	36±8
	5	37±8	39±7	38±8	37±8
	Mean	37±7	39±4	37±6	37±8
PND 38	1	34±3	36±3	35±4	37±4
	2	32±4	34±4	34±5	34±6

TABLE 13. Auditory startle latency to peak data (mean msec ±S.D.) [a]					
	Trial block	Dose (ppm)			
		0	0.5	2	8
	3	33±4	35±6	35±7	36±8
	4	35±8	36±5	37±8	38±6
	5	36±6	38±7	35±6	37±6
	Mean	34±4	36±4	35±5	36±4
PND 60	1	40±4	41±4	38±5	40±6
	2	36±6	39±5	35±3	37±5
	3	37±6	38±8	35±5	40±6
	4	39±8	40±7	35±5	40±6
	5	38±8	43±7	37±6	42±7
	Mean	38±5	40±4	36±3	40±5

[a] Data obtained from Tables 23-24, pages 213-224, MRID 45827601
N = 15-16/sex/dose

d. Learning and memory testing:

Passive avoidance: There were no treatment-related effects, and acquisition and retention were appropriate in control animals. Data are summarized in Table 14.

TABLE 14. Passive avoidance performance at PND 24/31(mean ± S.D.) [a]					
Test day/parameter		Dose (ppm)			
		0	0.5	2	8
Males					
Session 1 (Learning)	Trials to criterion	3.3±0.6	3.4±0.7	3.4±0.6	3.1±0.3
	Latency trial 1 (sec)	23.3±20.5	38.1±35.9	22.2±17.2	39.7±30.7
	Latency trial 2 (sec)	170.7±23.3	170.4±21.7	155.7±44.7	170.8±27.0
	Failed to Learn/No. Tested	0/16	0/16	0/16	0/16
Session 2 (Retention)	Trials to criterion	2.4±0.8	2.2±0.4	2.5±0.8	2.3±0.6
	Latency trial 1 (sec)	164.3±34.7	160.9±44.2	175.7±13.4	173.4±16.0
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	172.4±18.5	174.5±22.1
Females					
Session 1 (Learning)	Trials to meet criterion	3.3±0.6	3.3±0.6	3.20.4	3.1±0.3
	Latency trial 1 (sec)	20.7±16.2	18.6±15.3	26.5±19.6	24.7±31.1
	Latency trial 2 (sec)	173.5±18.7	168.2±29.6	171.8±19.7	171.0±33.8
	Failed to Learn/No. Tested	0/16	0/16	0/16	0/16
Session 2 (Retention)	Trials to meet criterion	2.2±0.4	2.4±0.7	2.3±0.6	2.5±0.7
	Latency trial 1 (sec)	165.8±40.7	157.9±49.4	159.4±46.2	166.6±39.6
	Latency trial 2 (sec)	180.0±0.0	175.4±14.8	175.7±17.2	166.8±36.4

[a] Data obtained from Table 25, pages 223-225, MRID 45827601.

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. Although there was no difference in the time to meet the criteria in learning between trial 1 and trial 2 for either males or females, it took the group fewer trials to meet the criteria in session 2 (retention) than in session 1 (learning) in males and females. It is noted however, that the number of trials to meet the criteria reflect only a slight learning during the trials (numerically different in some cases and not in others). This may indicate that the test used is too simple to adequately test for learning and retantion. Data are summarized in Table 15.

TABLE 15. Water maze performance

Test day/parameter		Dose (ppm)			
		0	0.5	2	8
Males					
Session 1 (Learning)	Trials to meet criterion	7.3±2.7	6.5±1.6	7.8±2.7	7.1±2.5
	Trial 1 errors (mean ± SD)	0.9±1.1	0.5±1.1	0.6±1.1	0.8±0.9
	Trial 1 duration (sec) (mean ± SD)	23.9±.20.6	15.4±10.6	14.3±12.0	17.1±10.4
	Trial 2 errors (mean ± SD)	0.7±0.9	0.6±1.1	0.8±0.9	0.7±1.1
	Trial 2 duration (sec) (mean ± SD)	20.6±19.5	14.7±14.2	19.8±15.5	16.4±15.5
	Failed to meet criterion	1/16 (6%)	0/16 (0%)	1/16 (6%)	0/16 (0%)
Session 2 (retention)	Trials to meet criterion	5.2±0.8	5.3±1.0	5.9±1.6	5.8±1.4
	Trial 1 errors (mean ± SD)	0.0±0.0	0.1±0.3	0.3±0.6	0.1±0.3
	Trial 1 duration (sec) (mean ± SD)	5.9±3.0	5.1±3.2	6.5±4.1	5.3±.2.8
	Trial 2 errors (mean ± SD)	0.0±0.0	0.0±0.0	0.1±0.3	0.1±0.5
	Trial 2 duration (sec) (mean ± SD)	3.4±1.1	3.3±0.6	3.9±1.6	4.8±2.7
Females					
Session 1 (Learning)	Trials to meet criterion	7.7±3.1	9.3±2.5	8.4±3.4	8.5±2.2
	Trial 1 errors (mean ± SD)	0.4±0.5	0.7±0.7	0.8±0.7	0.5±0.5
	Trial 1 duration (sec) (mean ± SD)	12.7±7.1	15.6±8.1	16.9±8.1	17.3±8.7
	Trial 2 errors (mean ± SD)	0.8±0.9	0.9±1.0	0.6±1.1	1.1±0.9
	Trial 2 duration (sec) (mean ± SD)	16.1±11.6	17.5±17.3	12.9±11.9	17.2±10.3
	Failed to meet criterion	1/15 (7%)	1/16 (6%)	1/14 (7%)	0/15 (0%)
Session 2 (retention)	Trials to meet criterion	7.4±3.2	8.0±3.5	6.4±2.3	7.0±3.4
	Trial 1 errors (mean ± SD)	0.4±0.9	0.7±1.0	0.4±0.9	0.3±0.5
	Trial 1 duration (sec) (mean ± SD)	11.4±10.7	13.4±9.0	9.5±6.9	7.9±7.6
	Trial 2 errors (mean ± SD)	0.2±0.6	0.3±0.5	0.0±0.0	0.1±0.3
	Trial 2 duration (sec) (mean ± SD)	6.0±4.9	7.5±6.3	3.6±1.4	3.9±1.8

[a]Data obtained from Table 26, pages 226-228. MRID 45827601.

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

5. Postmortem results:

a. **Brain weights:** No absolute brain weight differences were noted at any dose on any day measured. Mean brain weight data are presented in Table 16.

TABLE 16. Mean (±SD) Brain Weight Data in Offspring [a]

Parameter	Dose (ppm)			
	0	0.5	2	8
Males				
Day 21				
Terminal body weight (g)	51.6±5.0	47.7±3.5	49.3±4.8	42.4*±3.6
Brain weight (g)	1.464±0.072	1.397±0.087	1.411±0.082	1.392±0.047
Termination				
Terminal body weight (g)	331.8±22.2	319.7±22.8	318.2±23.5	317.4±30.2
Brain weight (g)	1.815±0.083	1.785±0.078	1.819±0.048	1.825±0.082
Females				
Day 21				
Terminal body weight (g)	50.1±4.5	48.5±4.9	45.7±3.9	39.4*±4.5
Brain weight (g)	1.388±0.070	1.397±0.061	1.375±0.071	1.332±0.069
Termination				
Terminal body weight (g)	201.9±9.7	190.7±16.5	189.6±20.8	184.6±13.1
Brain weight (g)	1.710±0.062	1.684±0.064	1.693±0.087	1.677±0.058

[a] Data obtained from pages 904-909, MRID 45827601. *p<0.05.
N = 9-10/sex/dose

b. **Cholinesterase activity:** Results of cholinesterase activity assessment are presented in Table 17. For the day 4 offspring, decreased cholinesterase activity was noted in only high-dose pups (pooled samples from males and females), with red blood cell cholinesterase activity being most affected. The observed decrease ($p \leq 0.05$) in brain cholinesterase in mid-dose pups was attributed to a low value in one pooled sample and is considered incidental to treatment. For the day 21 offspring, all three cholinesterase activities were decreased in high-dose males and females. Average decreases were 41-43% for plasma, 53-56% for red blood cell, and 30% for brain cholinesterase activities. Effects in the dams were briefly discussed above (Section II.A.4).

TABLE 17. Cholinesterase activity in dams and offspring

Cholinesterase [mean ± SD (% inhibition relative to control)]	Dose (ppm)			
	0	0.5	2	8
Dose in mg/kg/day during lactation	0	0.102	0.389	1.714
Dose in mg/kg/day during gestation	0	0.038	0.156	0.670
Lactation day 21 Dams				
Plasma (IU/mL)	0.72 ± 0.15	0.65 ± 0.17 (10)	0.28* ± 0.08 (61)	0.10* ± 0.05 (86)
RBC (IU/mL)	1.12 ± 0.18	0.82* ± 0.32 (27)	0.30* ± 0.18 (73)	0.16* ± 0.22 (86)
Brain (IU/g)	12.6 ± 0.7	11.4* ± 1.0 (10)	4.4* ± 0.4 (65)	1.8* ± 0.5 (86)
Day 4 Male & Female Offspring Combined				
Plasma (IU/mL)	0.62 ± 0.07	0.64 ± 0.9 (+3)	0.62 ± 0.07 (0)	0.55* ± 0.04 (-11)
RBC (IU/mL)	1.18 ± 0.20	1.19 ± 0.17 (+1)	1.22 ± 0.24 (+3)	0.59* ± 0.24 (-50)
Brain (IU/g)	4.4 ± 0.4	4.5 ± 0.6 (+2)	3.8* ± 0.7 (-14)	4.1 ± 0.2 (-7)
Day 21 Male Offspring				
Plasma (IU/mL)	0.61 ± 0.08	0.57 ± 0.08 (+7)	0.58 ± 0.08 (-5)	0.35* ± 0.13 (-43)
RBC (IU/mL)	1.43 ± 0.53	1.33 ± 0.38 (+7)	1.47 ± 0.25 (+3)	0.67* ± 0.26 (-53)
Brain (IU/g)	11.4 ± 0.4	11.4 ± 0.6 (0)	11.4 ± 0.4 (0)	8.0* ± 1.2 (-30)
Day 21 Female Offspring				
Plasma (IU/mL)	0.61 ± 0.09	0.64 ± 0.05 (+5)	0.55 ± 0.08 (-10)	0.36* ± 0.13 (-41)
RBC (IU/mL)	1.40 ± 0.23	1.18 ± 0.28 (-16)	1.32 ± 0.31 (-6)	0.62* ± 0.22 (-56)
Brain (IU/g)	11.5 ± 0.4	11.4 ± 0.4 (-1)	11.1 ± 0.5 (-3)	8.1* ± 1.2 (-30)

Data obtained from Appendix Tables, pages 869-875, MRID 45827601. Percentages from p. 52, MRID 45827601. *p≤0.05.

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 day 21 or study termination.

3. **Brain morphometry:** No treatment-related morphomeric effects were observed in any animals at PND 21 or study termination. Data are summarized in Table 18.

   Although the report indicated no treatment related morphometric effects were seen, data in Table 19 shows brain measurements that were statistically significantly increased over control values in postnatal day 70-80 in perfused male and female rats. The report author believed that these apparent deviations from control were not due to treatment because there was no corresponding brain microscopic pathology noted, there was no difference in these parameters in 21 day old rats, changes were small and there was no brain weight changes in these groups of rats. Consequently no measurements were reported on the mid and low dose levels.

TABLE 18. Mean (±SD) morphometric data in offspring [a]

Parameter	Dose (ppm)			
	0	0.5	2	8
Males				
Day 21				
Anterior to posterior cerebrum length (mm)	13.79±0.39	13.42±0.32	13.44±0.34	13.55±0.32
Anterior to posterior cerebellum length (mm)	7.24±0.45	7.34±0.25	7.47±0.31	7.01±0.31
Termination				
Anterior to posterior cerebrum length (mm)	14.76±0.53	14.82±0.39	14.77±0.25	14.90±0.47
Anterior to posterior cerebellum length (mm)	7.93±0.14	8.14±0.27	7.90±0.27	7.96±0.37
Females				
Day 21				
Anterior to posterior cerebrum length (mm)	13.49±0.21	13.44±0.28	13.56±0.37	13.34±0.27
Anterior to posterior cerebellum length (mm)	7.38±0.26	7.30±0.37	7.27±0.45	7.30±0.19
Termination				
Anterior to posterior cerebrum length (mm)	14.29±0.40	14.17±0.35	14.27±0.44	14.40±0.22
Anterior to posterior cerebellum length (mm)	7.54±0.33	7.74±0.34	7.69±0.39	7.72±0.25

a Data obtained from pages 904-909. MRID 45827601.
N = 9-10/sex/dose

Table 19: Statistically significant brain measurements in postnatal day 21-day old and 70-80 terminal rats .

Parameter	Dose level			
Dose in ppm	**0**	**0.5**	**2**	**8**
Dose in mg/kg/day (lactation)	0	0.102	0.389	1.714
Dose in mg/kg/day (gestation)	0	0.038	0.156	0.670
21-day old perfused male rats				
Frontal cortex	1.7526±0.01	NA	NA	1.8137±0.01
Parietal cortex	1.9145±0.01	NA	NA	1.8753±0.02
Caudate putamen	3.1729±0.02	NA	NA	3.1152±0.01
Corpus callosum	0.5289±0.01	NA	NA	0.4725±0.01
Hippocampal gyrus	1.4429±0.01	NA	NA	1.4939±0.02
Cerebellum	4.2263±0.17	NA	NA	4.3109±0.16
21-day old perfused female rats				
Frontal cortex	1.7489±0.07	NA	NA	1.8050±0.01
Parietal cortex	1.9070±0.01	NA	NA	1.8869±0.03
Caudate putamen	3.1117±0.04	NA	NA	3.1648±0.04
Corpus callosum	0.5096±0.003	NA	NA	0.4917±0.009
Hippocampal gyrus	1.5360±0.01	NA	NA	1.5480±0.01
Cerebellum	4.4939±0.03	NA	NA	4.4472±0.01
Postnatal day 70-80 perfused male rats				
Frontal cortex	1.6715±0.02	NA	NA	1.8155±0.03*
Parietal cortex	1.7404±0.01	NA	NA	1.8874±0.034*
Caudate putamen	3.3877±0.0075	NA	NA	3.4983±0.02*
Corpus callosum	0.5966±0.0065	NA	NA	0.5997±0.0065
Hippocampal gyrus	1.6484±0.0135	NA	NA	1.7256±0.04
Cerebellum	4.6742±0.16	NA	NA	4.3957±0.17
Postnatal day 70-80 perfused female rats				
Frontal cortex	1.6500±0.03	NA	NA	1.6912±0.019
Parietal cortex	1.6808±0.17	NA	NA	1.7420±0.02
Caudate putamen	3.3595±0.0091	NA	NA	3.4829±0.0067*
Corpus callosum	0.6588±0.009	NA	NA	0.6267±0.009
Hippocampal gyrus	1.5070±0.02	NA	NA	1.5606±0.01
Cerebellum	4.5182±0.12	NA	NA	4.3416±0.04

All three controls showed individual measurements overlapping with some individual measurements in the respective 8.0 ppm group. * Significant at $p<0.05$. Measurements in mm ± variance. NA Not available or submitted.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the overall NOEL is 0.5 ppm for dams based on slight inhibition of erythrocyte and brain cholinesterase activity on lactation day 21. The investigators also concluded that the overall NOEL for offspring is 0.5 ppm based on decreased body weight gain during lactation (both sexes), decreased post-weaning body weight (males only), and a marginal delay in vaginal patency at 2 ppm.

B. **REVIEWER COMMENTS:** In high-dose dams during gestation and lactation, treatment-related effects for maternal animals included repetitive chewing movements and muscle fasciculations which are consistent with signs of cholinergic toxicity and decreased cholinesterase activity. Body weight during lactation was decreased at each time point recorded for the high-dose group, with average differences of 5% on Lactation Day 0 and 8-9% thereafter, compared to controls. Because of the low magnitude of response (i.e., decreases less than 10%), the lack of dose response, and no decreases during the gestation period, the decreases seen at the high dose only during lactation were not considered to be adverse or of toxicological significance.

Cholinesterase activity was decreased ($p<0.05$) in maternal animals at 0.5, 2, and 8 ppm. Decreases were 10%, 27%, and 10% at 0.5 ppm, 61%, 73%, and 65% at 2 ppm, and 86%, 86%, and 86% at 8 ppm compared to control values for plasma, erythrocyte and brain cholinesterase activities, respectively.

In offspring, male and female body weights were significantly decreased at 8 ppm on lactation days 4, 11, 17 and 21. By postnatal day (PND) 28 post weaning, male pup weight was significantly decreased at 2.0 and 8.0 ppm and remained so until postnatal day 70. During this period female postnatal pup weight was decreased only at the highest dose tested (8.0 ppm). Preputial separation in males was unaffected by treatment. Although the onset of preputial separation was delayed an average of 1.1 days in high-dose animals, the effect is considered toxicologically insignificant as it falls within the historical control range. The mean age for attainment of vaginal opening for females was delayed in mid- and high-dose animals

No effects were seen in FOB, motor/locomotor, auditory startle/latency, learning and memory/latency tests or ophthamology. Habituation was noted in motor/locomotor and auditory startle tests. Absolute brain weights were unchanged. Perfused 21 day old brains showed no difference from control values and the groups showed no difference from controls in absolute brain weight. Morphometric data on perfused brains showed no definitive effects. The report indicated that the forebrain frontal and parietal cortex were statistically significantly increased in postnatal day 70-80 males and that the forebrain caudate putamen in postnatal males and females were statistically significantly increased at 8.0 ppm. Since the report author did not believe the measurements were treatment related, the corresponding mid and low dose values were not reported. The author did not believe that the values were treatment related because of the low magnitude in response and lack of corresponding microscopic findings.

Statistically significant decreases in cholinesterase activity were seen in the day 4 d male and female (combined) pups as well as in the Day 21 male and female pups On PND day 4. offspring showed statistically significant decreases in plasma (11%) and erythrocyte (50%) cholinesterase inhibition at 8 ppm. On PND 21, male/female pups showed cholinesterase inhibition in plasma (43%/41%), erythrocyte (53%/56%) and brain (30%/30%) only at 8.0 ppm.

There was a non-dose related but statistically significant decrease in brain cholinesterase activity at 2 ppm only. This decrease was attributed to a low value in one pooled sample and is considered incidental to treatment.

For maternal systemic toxicity, the LOAEL is 8 ppm (0.670 mg/kg/day) based on cholinergic signs. The maternal systemic NOAEL is 2 ppm (0.156 mg/kg/day) in the diet.

For maternal cholinesterase inhibition, the LOAEL is 0.5 ppm (0.038 mg/kg/day) based on statistically significant decreases in erythrocyte and brain cholinesterase activities. The maternal cholinesterase inhibition NOAEL was not determined.

For offspring systemic toxicity, the LOAEL is 2 ppm (0.156 mg/kg/day) based on delayed vaginal opening in females. The offspring systemic toxicity NOAEL is 0.5 ppm (0.038 mg/kg/day).

For offspring cholinesterase inhibition, the LOAEL is 8 ppm (0.670 mg/kg/day) based on decreased plasma and erythrocyte cholinesterase activity on PND 4 (male and female pups combined) and decreased cholinesterase activity in all three compartments in male and female pups on PND 21. The offspring cholinesterase inhibition NOAEL is 2 ppm (0.156 mg/kg/day).

C. **STUDY DEFICIENCIES:** None

13544

R113179

Chemical:	**Disulfoton**
PC Code:	**032501**
HED File Code	**13000 Tox Reviews**
Memo Date:	**08/18/2005**
File ID:	**TX0051561**
Accession Number:	**412-05-0100**

HED Records Reference Center
08/25/2005