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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

April 17, 2007
TXR # 0052894

SUBJECT: ETHOPROP (Ethoprophos). Review of Developmental Neurotoxicity Study.
DP Barcode: D309294
PC Code: 041101

FROM: Kit Farwell, D.V.M.
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THROUGH: Elizabeth Mendez, Ph.D.
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I. CONCLUSIONS

A developmental neurotoxicity study with ethoprop (ethoprophos) has been reviewed (MRID 46364801 and 46364802). This study is classified Acceptable/Non Guideline and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats pending comprehensive review of the positive control data.

II. BACKGROUND

This study was submitted in response to the data call in for developmental neurotoxicity studies for organophosphate pesticides.

III. RESULTS

The maternal LOAEL for Ethoprop in rats is 180 ppm (38.2 mg/kg/day during lactation) based on clinical signs (coarse tremors, repetitive chewing, muscle fasciculations, and nasal stains) and decreased body weight and body weight gain during lactation. The maternal NOAEL is 30 ppm (6.2 mg/kg/day during lactation).

The offspring LOAEL for Ethoprop in rats is 3 ppm (0.7mg/kg/day), the lowest dose tested, based on increased motor activity in male pups on PND 17. The offspring NOAEL is not established.

On LD 21, dams treated with 180 ppm had marked inhibition of plasma ChE (89%), RBC AChE (90%) and brain AChE (85%) activities in relation to control values. At 30 ppm, plasma ChE (77%), RBC AChE (85%) and brain AChE (49%) activity inhibition were reported. Inhibition of plasma ChE (34%) and RBC AChE (30%) activity occurred at 3 ppm; no inhibition was seen in brain AChE activity.

Pooled male and female offspring blood samples were tested on PND 4. In offspring, only plasma ChE (22%) activity was significantly inhibited at 180 ppm. RBC AChE was non-significantly decreased (12%) at 180 ppm. On PND 21 in male and female offspring, plasma ChE (68-71%), RBC AChE (62-76%) and brain AChE (50-62%) ChE activities were significantly inhibited at 180 ppm. At 30 ppm, plasma ChE (32-40%) and brain AChE (7-10%) activities were significantly inhibited in both sexes.

The maternal LOAEL for plasma ChE and RBC AChE inhibition for Ethoprop in rats is 3 ppm (0.7 mg/kg/day during lactation), the lowest dose tested, based on enzyme inhibition on LD 21. The maternal NOAEL for plasma ChE and RBC AChE inhibition is not established.

The maternal LOAEL for brain AChE inhibition for Ethoprop is 30 ppm (6.2 mg/kg/day during lactation) based on enzyme inhibition on LD 21. The maternal NOAEL for brain AChE inhibition is 3 ppm (0.7 mg/kg/day during lactation).

The offspring LOAEL for RBC AChE inhibition for Ethoprop in rats is 180 ppm (38.2 mg/kg/day during lactation, respectively) based on enzyme inhibition on PND 21. The offspring NOAEL for RBC AChE inhibition is 30 ppm (6.2 mg/kg/day during lactation).

The offspring LOAEL for plasma ChE and brain AChE inhibition for Ethoprop in rats is 30 ppm (6.2 mg/kg/day during lactation) based on enzyme inhibition on PND 21. The offspring NOAEL for plasma ChE and brain AChE inhibition is 3 ppm (0.7 mg/kg/day during lactation).

IV. DISCUSSION

This study is consistent with previously conducted toxicity studies with ethoprop and should have no effect upon the ethoprop risk assessment because NOAEL and LOAEL values in this study are higher than NOAELs and LOAELs selected for points of departure and endpoints in the ethoprop risk assessment.

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DATA EVALUATION RECORD

ETHOPROP

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRIDS 46364801 (main study), 46364802 (preliminary study)

Prepared for

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4/19/07

TXR#: 0052894

DATA EVALUATION RECORD

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

PC CODE: 041101 **DP BARCODE:** D309294

TEST MATERIAL (PURITY): Technical Grade Ethoprophos (93.6%)

SYNONYMS: Ethoprop; O-Ethyl S,S-dipropyl phosphorodithioate

CITATION: Sheets, L.P.; Fickbohm, B. (2004) A developmental neurotoxicity screening study with technical grade ethoprophos in Wistar rats. Bayer CropScience LP Toxicology, Stilwell, KS. Study Number 03-D72-PQ; September 10, 2004. MRID 46364801. Unpublished

Sheets, L.P. (2004) Maternal and fetal cholinesterase activities in Wistar rats following dietary exposure during gestation to technical grade ethoprophos. Bayer CropScience LP Toxicology, Stilwell, KS. Study Number 02-D72-NN; September 10, 2004. MRID 46364802. Unpublished

SPONSOR: Bayer CropScience

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46364801), Ethoprop (93.6% a.i., batch # OP 9950044) was administered in the diet to 30 female mated Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR rats/dose at nominal concentrations of 0, 3, 30 and 180 ppm from gestation day (GD) 6 through lactation day (LD) 21. Average doses to the animals were 0, 0.3, 2.8 and 16.6 mg/kg/day during gestation and 0, 0.7, 6.2 and 38.2 mg/kg/day during lactation for the 0, 3, 30 and 180 ppm groups, respectively.

A Functional Operational Battery (FOB) was performed on 30 dams/dose on GDs 13 and 20, and on 10 dams/dose on LDs 11 and 21. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (passive avoidance and watermaze testing), and neuropathology at study termination (day 75±5 of age). On PND 21, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Brain and erythrocyte (RBC)

acetylcholinesterase (AChE) and plasma cholinesterase (ChE) activities were measured in offspring (10/dose group) on PNDs 4 and 21 and in dams (10/dose group) on LD 21. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No parental animals died during gestation or lactation. No treatment-related clinical signs of toxicity were observed during gestation. During lactation, females at 180 ppm had an increased incidence of coarse tremors (7), repetitive chewing movements (7), muscle fasciculations (1), and nasal stains (2), none of which were observed in the control or other treated groups. During the FOB, the dams at 180 ppm had an increased incidence of tremors (2) and red nasal stains (1) on LD 11 and tremors (4), repetitive chewing (2), and brown perianal stains (1) on LD 21 compared with no abnormal findings in the controls. Mean body weight and food consumption were not affected by treatment during gestation. Mean body weight gain in females at 180 ppm was non-significantly decreased (90% of control value) for GDs 6-20. During lactation mean body weight for the high-dose dams was non-significantly decreased (94% of control value) on LD 4 and significantly decreased on LDs 7 through 21 (90-92% of control value). Body weight gain in females at 180 ppm was decreased (45% of control value) during the lactation period. Food consumption in high-dose females was non-significantly decreased (92-94% of control value) during the second and third weeks of lactation.

No treatment-related effects were observed on reproduction parameters. There was no treatment-related effect on the mean number of delivered pups per dam and survival during lactation and post-weaning was comparable between control and treated groups.

Mean body weight of offspring in treated and control groups was similar at parturition but was significantly decreased in males (82-84% of control value) and females (81-85% of control value) at 180 ppm beginning on PND 11. Mean body weight gain was significantly decreased in males (79-85% of control value) and females (78-84% of control value) at 180 ppm throughout lactation. Post-weaning (PNDs 28-70) body weight was significantly decreased in males (87-94% of control value) and females (89-94% of control value) at 180 ppm. The mean age at sexual maturation was not affected in either sex by treatment.

FOB parameters, auditory startle reflex habituation, and learning and memory (passive avoidance) were comparable between treated and control offspring. An effect on learning (water maze) in high-dose males was noted as an increase in the number of trials to criterion and a non-statistically significant increase in the trial 2 duration. Motor activity in all male treatment groups was increased on PND 17 due to a lack of habituation. Mean absolute brain weight was unaffected by treatment. No treatment-related findings were observed on gross or microscopic examination and morphometrics of the nervous system.

The maternal LOAEL for Ethoprop in rats is 180 ppm (38.2 mg/kg/day during lactation) based on clinical signs (coarse tremors, repetitive chewing, muscle fasciculations, and nasal stains) and decreased body weight and body weight gain during lactation. The maternal NOAEL is 30 ppm (6.2 mg/kg/day during lactation).

The offspring LOAEL for Ethoprop in rats is 3 ppm (0.7mg/kg/day), the lowest dose tested, based on increased motor activity in male pups on PND 17. The offspring NOAEL is not established.

On LD 21, dams treated with 180 ppm had marked inhibition of plasma ChE (89%), RBC AChE (90%) and brain AChE (85%) activities in relation to control values. At 30 ppm, plasma ChE (77%), RBC AChE (85%) and brain AChE (49%) activity inhibition were reported. Inhibition of plasma ChE (34%) and RBC AChE (30%) activity occurred at 3 ppm; no inhibition was seen in brain AChE activity.

Pooled male and female offspring blood samples were tested on PND 4. In offspring, only plasma ChE (22%) activity was significantly inhibited at 180 ppm. RBC AChE was non-significantly decreased (12%) at 180 ppm. On PND 21 in male and female offspring, plasma ChE (68-71%), RBC AChE (62-76%) and brain AChE (50-62%) activities were significantly inhibited at 180 ppm. At 30 ppm, plasma ChE (32-40%) and brain AChE (7-10%) activities were significantly inhibited in both sexes.

The maternal LOAEL for plasma ChE and RBC AChE inhibition for Ethoprop in rats is 3 ppm (0.7 mg/kg/day during lactation), the lowest dose tested, based on enzyme inhibition on LD 21. The maternal NOAEL for plasma ChE and RBC AChE inhibition is not established.

The maternal LOAEL for brain AChE inhibition for Ethoprop is 30 ppm (6.2 mg/kg/day during lactation) based on enzyme inhibition on LD 21. The maternal NOAEL for brain AChE inhibition is 3 ppm (0.7 mg/kg/day during lactation).

The offspring LOAEL for RBC AChE inhibition for Ethoprop in rats is 180 ppm (38.2 mg/kg/day during lactation, respectively) based on enzyme inhibition on PND 21. The offspring NOAEL for RBC AChE inhibition is 30 ppm (6.2 mg/kg/day during lactation).

The offspring LOAEL for plasma ChE and brain AChE inhibition for Ethoprop in rats is 30 ppm (6.2 mg/kg/day during lactation) based on enzyme inhibition on PND 21. The offspring NOAEL for plasma ChE and brain AChE inhibition is 3 ppm (0.7 mg/kg/day during lactation).

Doses during lactation were selected for the NOAEL and LOAEL, rather than lower doses during gestation, because effects were seen during lactation and not during gestation. It should be noted that several specifications of the DCI are not adequately addressed in the current protocol. The major inadequacy is a lack of information regarding duration of exposure and dose to the pups.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. **It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending comprehensive review of the 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:** Technical grade Ethoprophos (Ethoprop)

Description:	Colorless liquid
Batch #:	OP 9950044
Purity:	93.6 % a.i.
Compound Stability:	expiration date September 2004
CAS # of TGAI:	13194-48-4

2. **Vehicle**: acetone used as solvent to dissolve test material

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

Species:	Rat	
Strain:	Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR	
Age at study initiation:	Males: 15 weeks; females: 12 weeks	
Wt. at study initiation:	Females: approximately 180-278 g (GD 6)	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Males and females in individual stainless steel cages, except during co-habituation; individual dams and litters in plastic cages during gestation and lactation; littermates together in plastic cages for one week after weaning, then individually in stainless steel 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/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Six days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: May 5, 2003; End: August 15, 2003

2. **Study schedule:** Mated female Wistar rats (30/dose group) were administered the test material in the diet from gestation day (GD) 6 through lactation day (LD) 21. On postnatal day (PND) 4, litters were standardized to 8 pups, sexes were represented as equally as possible. Pups were weaned from their dam on PND 21 but were not treated with test material. Dams were sacrificed after weaning. Pups remained on study to PND 75.

3. **Mating procedure:** One male and one female were co-housed for a maximum of five consecutive days. The day that a vaginal plug or sperm in a vaginal smear was observed was designated gestation day (GD) 0.

4. **Animal assignment:** After the acclimation period, dams with body weight more or less than 20% of the mean weight were rejected and sacrificed without necropsy. The remaining females were assigned to the control or treated groups in sequence as they were determined to be inseminated, as shown in Table 1. Parental generation males were only breeders and were arbitrarily selected for co-housing with females.

TABLE 1. Study design				
Experimental parameter	**Dietary concentration (ppm)**			
	0	**3**	**30**	**180**
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	30	30	30	30
FOB (GDs 13 and 20)	30	30	30	30
FOB (LDs 11 and 21)	10	10	10	10
Offspring				
	Minimum No. of offspring assigned[a]			
Set A - motor activity (PNDs 13, 17, 21 and 60±2)[a]	10/sex	10/sex	10/sex	10/sex
Set B - Acoustic startle habituation (PND 22, 38±2 and 60±2)[a]	10/sex	10/sex	10/sex	10/sex
Set C - Passive Avoidance (PNDs 22, 29), Water Maze (PND 60±2, 67±2), FOB (PNDs 4, 11, 21, 35±1, 45±1, 60±2)[a]	10/sex	10/sex	10/sex	10/sex
Set D - Tissues (PND 21), Cholinesterase (PND 21)	10/sex	10/sex	10/sex	10/sex
Set A-C - Ophthalmology (PND 50-60), tissues (PND 75±5), brain weight (PND 75±5)	10/sex	10/sex	10/sex	10/sex

[a] One male and/or female per litter (approximately 16/sex, representing 20 litters per dose) with a minimum of 10/sex were assigned.

5. **Dose selection rationale:** The rationale for dose selection was based on the results of the two-generation reproduction study (MRID 41921201) and a preliminary study intended to determine the levels of cholinesterase (ChE) inhibition in dams and fetuses after dietary exposure from GD 6 through GD 20 (MRID 46364802).

   In the reproduction study, technical grade ethoprop was administered in the diet to Sprague-Dawley rats at concentrations of 0, 1, 30 and 300/150 ppm. The 300 ppm level was reduced to 150 ppm one week after weaning of the F_{1A} offspring due to pup mortality. The high dose parental generation (F_0) animals received the 150 ppm concentration for three weeks before mating to produce the F_{1B} generation and for the remainder of the study. The high-dose F_0 females had soft feces during the first two weeks of exposure (premating) as well as tremors during gestation and lactation. For the high-dose F_0 females, body weight gain and food consumption were decreased for the F_{1A} gestation period and body weight was less than that of the controls during the F_{1A} lactation period. Body weight was also reduced for the high dose F_1 females during lactation. Plasma and brain ChE activity were inhibited 97% and 47%, respectively, in high dose F_0 females; RBC ChE activity was not affected. At 30 ppm in the F_0 generation females, plasma and brain ChE activity were inhibited 90% and 19%, respectively.

   The results of the preliminary study (MRID 46364802) are discussed in the Appendix to this DER.

6. **Dosage administration:** Ethoprop was administered in the diet to maternal animals on GD 6 through LD 21. After PND 21, untreated food was provided for all groups.

7. **Dosage preparation and analysis:** Acetone was used to dissolve the test material for mixing in the diet. The acetone was allowed to evaporate before the feed was provided to the animals. The control diet was prepared in the same way but without the test material. The diet with test material was prepared weekly and stored at freezer temperature until presentation to the animals. Concentrations of the test substance in the diet were measured for weeks 1 through 5 of the study. The stability and homogeneity of the test material in the diet were previously verified at dietary concentrations of 3 and 240 ppm.

Results:

Homogeneity analysis: Concentrations of nine samples each of diets containing 3 and 240 ppm were 96-109% and 97-104% of the nominal concentrations, respectively, with relative standard deviations (RSD) of 3.46 and 2.37%, respectively.

Stability analysis: After seven days at room temperature, there was a 3% and 6% decline in the 3 and 240 ppm concentrations, respectively.

Concentration analysis: Mean values for weeks 1-5 were 109, 107 and 104% of the 3, 30 and 180 ppm concentrations, respectively, with a RSD of 4.10-7.89%.

The analytical data indicated that the homogeneity, concentration and stability of ethoprop in the diet preparations were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Females were observed cage-side for mortality, moribundity and clinical signs of toxicity at least once daily during the study. A physical examination was performed once daily during the dosing period.

Those females presumed to be pregnant (approximately 30 per group) were observed outside the home cage for a functional observational battery (FOB) at least twice during the gestation period (days 13 and 20). At least 10 dams per group were examined twice during the lactation period (days 11 and 21). The examiner was unaware of the animal's group assignment. The arena size and examination details were not provided. 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 exophthalmos; 3) Ranking or count of urination and defecation, including polyuria and diarrhea; 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size; 5) Degree of palpebral closure, e.g., ptosis; 6) Respiration; 7) Activity/arousal level.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded on GDs 6, 13 and 20 and LDs 0, 4, 7, 14 and 21. Food consumption was measured GDs 6, 13 and 20 and LDs 0, 7, 14 and 21.

Blood samples were collected from the orbital plexus from approximately 10 dams per group on LD 21 for plasma ChE and red blood cell acetylcholinesterase (AChE) activity measurements. Brains were collected for AChE activity assays immediately after the blood collection. Animals were not fasted prior to sample collection. The ChE activity methodology is described later in this DER.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as PND 0. The number of pups delivered and the pup status at birth were recorded for each litter. If a dam delivered less than three pups per sex or if the litter size decreased to less than seven pups by PND 4, the dam and litter were sacrificed without necropsy. All pups were observed cage-side once daily for mortality, moribundity, clinical signs of toxicity and behavioral changes. Detailed observations for clinical signs were made once daily before weaning and once weekly thereafter.

   On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible) using a random selection technique. If there were more than 23 acceptable litters for any group, the surplus litters were sacrificed on PND 4 after weighing and without necropsy. Preference was given to retaining litters with four males and four females. Culled dams and pups and dams with insufficient litters were sacrificed by CO_2 asphyxiation and decapitation, respectively.

   Surviving pups were weighed on PND 0, 4, 11, 17 and 21 and once weekly thereafter. They were also weighed when vaginal patency and preputial separation were first evident. Food consumption was not measured after weaning on PND 21.

2) **Developmental landmarks:** Beginning on postnatal day 38, male offspring were examined daily for preputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset and the offspring body weight at that time were recorded. All pups were also tested for pupil constriction on PND 21.

3) **Postweaning observations:** After weaning on PND 21, offspring were subjected to cage-side observations once daily and detailed weekly observations. Individual offspring body weight data were recorded weekly.

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

I) **Functional observational battery (FOB) (Set C):** On PNDs 4, 11, 21, 35±1, 45±1, and 60±2, a minimum of ten offspring/sex/group representing at least 20 litters/group were examined outside the home cage in an FOB assessment by an individual who was unaware of the group assignment. The same parameters assessed in the maternal FOB were examined in offspring, as appropriate for the developmental stage being observed. Neonates (PNDs 4 and 11) were not evaluated in the open field since the observer did not consider it necessary for the evaluation.

ii) **Motor activity testing (Set A):** Motor activity was evaluated in a minimum of ten pups/sex/dose representing at least 20 litters/group on PNDs 13, 17, 21 and 60±2. Activity was monitored for 60 minutes (six ten-minute intervals) in figure-eight mazes. Each maze consisted of eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys). A Columbus Instruments Universal Maze Monitoring System was used for data collection. Broad-spectrum background noise was provided throughout the test to minimize acoustical variation during testing. The uniformity of light intensity over each maze was verified each day. Motor activity was measured as the number of beam interruptions that occurred during each session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of each session.

iii) **Auditory startle reflex habituation (Set B):** Auditory startle reflex habituation testing was performed on at least ten offspring/sex/dose representing at least 20 litters per group on PNDs 22, 38±2 and 60±2. Groups of four animals were tested simultaneously within the startle system enclosure. The enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material and housed a speaker mounted in the ceiling to provide the eliciting stimulus (a 50-msec burst of white noise at approximately 118 dB). The enclosure housed four load cell/force transducer assemblies that measured the startle response. During the test session, the animals were placed in cages that were positioned on the top of each load cell. Sound measurements were made using a Bruel and Kjaer Real-Time Frequency Analyzer fitted with a microphone. The animals were allowed a 5-minute acclimation period in the enclosure at ambient noise levels before being presented with the startle-eliciting stimulus at 10-sec intervals. Data collection began with the presentation of the stimulus and continued for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (one sample/msec for 200

msec) and converted to grams using a calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's response curve. Baseline was defined as the average force (g) exerted on the platform during the first eight msec following the onset of the stimulus. This baseline was taken to represent an approximate body weight measurement to verify that the equipment was functioning properly. Response amplitude was defined as the maximum value of the average curve minus the baseline. Latency to peak was the time (msec) following the onset of the stimulus when the peak response occurred.

iv) **Learning and memory testing (Set C):** Learning, short-term retention and long-term retention were assessed in a **passive avoidance test** on PNDs 22 and 29. A minimum of ten pups/sex/group representing at least 20 litters/group were tested. Only animals that demonstrated acquisition were tested for retention. Testing was conducted using an integrated system of equipment and computer programs from Coulbourn Instruments. Testing occurred in individual isolation cubicles, each with a single shuttle cage. Each shuttle cage (approximately 14 x 7 inches) was separated into two compartments of equal size by a wall that supported a centrally-located sliding door. The walls of one compartment were lined with black film (dark side) and the walls of the other compartment were illuminated with a high-intensity lamp. After adaptation, the animal was placed into the lighted compartment facing toward the light. After approximately 20 seconds, the light was illuminated and the door between the compartments was opened. When the rat moved into the dark compartment, the door closed, a shock delivered and the light switched off. The rat was then returned to its cage until the next trial. If the rat did not cross to the dark compartment within 180 sec, it was returned to its cage and given a latency score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials elapsed, whichever occurred first. Animals that failed to reach criterion performance with 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase. The test was repeated one week later. In the second trial, rats were placed in the illuminated compartment, given a 20-sec acclimation period and then the latency to enter the dark side was recorded. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Learning and memory in a minimum of 10 animals/sex/group representing at least 20 litters per group were also assessed using **water maze testing** on PND 60±2 and again seven days later. The M-maze was constructed of Plexiglas with five inch wide corridors and approximately 7.5 inches of water maintained at 22±1^{0}C. For each trial, the rat was placed in the starting position at the base of the M-maze stem, located between the two lateral arms. For the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the maze. The initial arm for the learning trial was the incorrect goal for the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any trial were guided to the correct goal with the exit ramp and removed from the maze. Between trials, the animals were kept in a transport cage for approximately 15±5 seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in a test session was fifteen. Latency (time in

seconds to choose the correct goal or the maximum of 60 seconds) and the number of errors (incorrect turns in the maze) were recorded. Only animals that demonstrated acquisition within the 15-trial limit were tested for retention seven days later. Dose groups were compared using the following measures:

Acquisition (First Test): number of trials to criterion (measure of overall learning); average number of incorrect turns in maze for each trial (measure of overall learning); and latency to reach the correct goal on trial 2 (measure of short-term retention).

Retention (Second Test): number of trials to criterion (measure of long-term retention); average number of errors for each trial (measure of long-term retention); and latency to reach the correct goal on trial 1 (measure of long-term retention).

5) **Ophthalmology**: At approximately 50-60 days of age, ophthalmic examinations were conducted on a minimum of 10 rats/sex/group representing at least 20 litters selected for perfusion at study termination. Pupillary reflex was tested using a penlight or transilluminator after dilation of the pupils with a mydriatic. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilation. After dilation, the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

6) **Cholinesterase determination:** Brain and RBC AChE and plasma ChE activities were determined in offspring on PNDs 4 and 21 and in dams on LD 21. On PND 4, blood samples were collected from a minimum of 10 pups/sex/group randomly selected for culling. Samples from male and female pups within the same litter were pooled (generally blood from at least four pups). Blood samples were collected from a minimum of 10 dams/group on LD 21 and 10 pups/sex/group representing 20 litters from Set D on PND 21. Blood was collected via decapitation (PND 4 pups) or via the orbital plexus (dams and PND 21 pups). The brain was collected immediately after the blood collection. Blood and brain ChE activity analyses were conducted with a modified Ellman method in which 6, 6'-dithiodinicotinic acid was used as a coupling agent and a 340 nm wavelength for the spectrophotometer.

2. **Postmortem observations**:

a. **Maternal animals:** Parental females found moribund were sacrificed by CO_2 asphyxiation. Females found dead or moribund were subjected to a gross necropsy examination and possible collection of tissues at the discretion of the study director.

Following co-habituation, males were sacrificed and discarded. Parental dams were sacrificed by CO_2 asphyxiation on LD 21 after the weaning of their litters; a necropsy was not conducted. Mated females that did not deliver a litter were sacrificed on GD 24 without a necropsy.

b. **Offspring**: All moribund pups were sacrificed and subjected to a gross necropsy examination. Tissues were collected at the discretion of the study director. Animals found dead underwent a necropsy and were disposed of without collection of tissues.

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Pups selected for culling but not required for ChE activity assays were sacrificed by decapitation and discarded without necropsy.

Animals selected for perfusion on PND 21 (Set D) and at study termination (Sets A-C) were anaesthetized with an intraperitoneal dose of pentobarbital and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by *in situ* fixation using universal fixative [1% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde] in phosphate buffer. On PND 21, only the brain (with olfactory bulb) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves) and selected bilateral peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. All tissues were placed in 10% buffered formalin. The brain was weighed upon removal from the skull before placement in the formalin and the brain: body weight ratio was calculated.

Prior to sectioning for histology, the following brain measurements were made using a Vernier caliper: 1) anterior-to-posterior length of the cerebrum, extending from the anterior pole to posterior pole, exclusive of the olfactory bulbs; and 2) anterior-to-posterior length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole. These measurements were performed by a technician who was aware of the dose assignments.

After the gross measurements, the brain was divided into eight coronal sections for microscopic examination. The eight sections were processed for paraffin embedding, sectioned and examined after staining with hematoxylin and eosin (H&E). Brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained with luxol fast blue/cresyl violet. The following tissues were also collected from the perfused animals at the terminal necropsy for embedding in paraffin and staining with H&E: three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA), sectioned and stained with a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

Only tissues from control and high-dose animals were examined for micropathology and morphometry. If no lesions were found, the other dose groups were not examined. Sections were coded and examined in randomized order without knowledge of the code. The frequency and severity of each lesion was determined before the code was broken and the data evaluated for a dose-effect relationship.

Eight linear measurements of selected brain regions were taken, including the two gross measurements on the intact brain discussed above. The other six taken from the histologic sections using software calibrated with an ocular micrometer were as follows:

1. Frontal cortex thickness (forebrain) - measurement of the dorsal portion of the cerebral cortex within a coronal section passing through the region of the optic chiasm.

2. Parietal cortex thickness (forebrain) - measurement of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. Caudate putamen horizontal width (forebrain; maximum cross-sectional width) - measurement on the coronal section at the level of the optic chiasm.

4. Corpus callosum thickness (forebrain) - measurement at the mid-point of this region, within the section taken at the level of the optic chiasm.

5. Hippocampal gyrus thickness (midbrain) - mean of two measurements of the full width on both sides of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter.

6. Cerebellum height (cerebellum/pons) - measurement extending from the roof of the fourth ventricle to the dorsal surface.

All brain sections from the control and high-dose male and female offspring also underwent a micropathologic evaluation.

D. DATA ANALYSIS:

1. **Statistical analyses:** Continuous data were assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. If there were unequal variances, the data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons).

   FOB continuous data were analyzed using an ANOVA, with *post-hoc* comparisons using Dunnett's test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling Procedures, with *post-hoc* comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

   Motor and locomotor activity (total session activity and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data for the four test occasions were analyzed using an ANOVA to determine if there was a significant day-by-treatment interaction. If so, Dunnett's test was used to determine if the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine if there was a significant treatment-by-interval interaction on each test occasion. If so, the data were analyzed using Dunnett's test to determine whether the treated group was significantly different from the control.

   Acoustic startle response amplitude data (peak amplitude) for the first three occasions were first analyzed using an ANOVA. If there was a significant group effect, Dunnett's test was used to determine if the treated group was significantly different from the control. The response amplitude data for each block of ten trials (five blocks/test session) were analyzed

using a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group-by-block interaction, the block values were analyzed using Dunnett's test to determine if the treated group was significantly different from the control.

Passive avoidance latency data were analyzed using a Wilcoxon Test for time to failure. The number of trials to criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats not meeting the criterion level in the learning phase was analyzed as incidence data.

Water maze latency data were analyzed by a univariate ANOVA, with *post-hoc* analysis using Dunnett's test. The number of trials to criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats not meeting the criterion level in the learning phase was analyzed as incidence data.

Pathology data were screened for potential effects and then evaluated using the table below.

Data Type	Data	Statistical Tests
	Cholinesterase Levels	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Organ Weight	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Gross Brain Measurements	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Microscopic Brain Measurements	ANOVA and/or T-test (2)
Frequency	Ophthalmology	Visually Screened (3)
	Gross Pathology	Visually Screened (3)
	Micropathology	Chi-Square Fisher's Exact Test

(1) ANOVA was used if data were homogeneous; Kruskal-Wallis was used if data were nonhomogeneous.
(2) A T-test, 2-tailed, was used for two-group comparisons; an ANOVA was used for multiple-group comparisons.
(3) If potential compound effects were suspected, then a Chi-square and one-tailed Fisher's Exact test were used.
All statistical tests used a significance level of $p \leq 0.05$, except for Bartlett's test, which used $p \leq 0.01$.

2. Indices:

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

$$\text{Mating index (\%)} = \frac{\text{number of inseminated females}}{\text{number of females cohoused with males}} \times 100$$

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

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

$$\text{Live birth index (\%)} = \frac{\text{number of live born pups at birth per litter}}{\text{total number of pups born per litter}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{number of live pups on day 4 preculling per litter}}{\text{number of live pups born per litter}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{number of live pups on day 21 per litter}}{\text{number of live pups on day 4 post-culling per litter}} \times 100$$

3. **Positive and historical control data:** Positive control data are presently under review

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No parental females died or were sacrificed moribund during the study.

   Clinical signs in parental females reported from cage-side observation during gestation were unrelated to treatment. Signs included exophthalmia (one high-dose), areas of hair loss (two at each dietary level) and scab formation (one control). During lactation, treatment-related clinical signs in high-dose females included coarse tremors, repetitive chewing movements and muscle fasciculations which began on LD 5-7 and increased in incidence through lactation (except for muscle fasciculations) . Two animals with tremors and repetitive chewing movements also had nasal stains. Selected clinical signs reported during cage-side observations in lactation are summarized in Table 2.

   All females presumed pregnant on GDs 13 and 20 and randomly selected subsets of 10 dams per group with acceptable litters on LDs 11 and 21 were observed during FOB evaluation. However, only nine females in the high-dose group underwent FOB assessment on LDs 11 and 21. Treatment-related clinical signs observed in high-dose females during lactation included tremors, repetitive chewing movements, nasal stain and perianal stain. Other signs observed during gestation and lactation included significantly decreased incidence of minimal resistance to cage removal (high-dose dams on GD 13 and 20, low-dose dams on GD 20), significantly increased number of rearings (mid-dose females on GD 13) and exophthalmia (one high-dose dam on all days). Selected clinical signs reported during FOB observations are summarized in Table 3.

TABLE 2. Maternal cage-side clinical observations during lactation (total number of animals affected)[a]

Observation	Dietary concentration (ppm)			
	0	3	30	180
Lactation				
Coarse tremors	0	0	0	7
Repetitive chewing movement	0	0	0	7
Muscle fasciculation	0	0	0	1
Nasal stain	0	0	0	2

[a] Data obtained from pages 85-86, MRID 46364801.
N=20-30

TABLE 3. Maternal FOB clinical observations during gestation and lactation (number of animals affected)[a]

Observation	Dietary concentration (ppm)			
	0	3	30	180
Gestation				
GD 13				
Ease of removal - number examined	30	30	30	30
Minimal resistance	24	18	20	14*
Minimal resistance with vocalization	6	12	10	16*
Rearing - number examined	30	30	30	30
Mean ± S.D.	2.4 ± 0.9	3.0 ± 1.0	3.1* ± 1.2	2.4 ± 1.2
GD 20				
Ease of removal - number examined	30	30	30	30
Minimal resistance	22	13*	20	14*
Minimal resistance with vocalization	8	17*	10	16*
Lactation				
LD 11				
Stains - Nasal - number examined	10	10	10	9
Not observed	10	10	10	8
Red	0	0	0	1
Tremors - number examined	10	10	10	9
Not observed	10	10	10	7
Moderate to severe	0	0	0	2
LD 21				
Stains - Perianal - number examined	10	10	10	9
Brown				
Not observed	10	10	10	8
Moderate to severe	0	0	0	1
Repetitive Chewing - number examined	10	10	10	9
Not observed	10	10	10	7
Slight	0	0	0	1
Moderate to severe	0	0	0	1
Tremors - number examined	10	10	10	9
Not observed	10	10	10	5*
Slight	0	0	0	1
Moderate to severe	0	0	0	3

[a] Data obtained from pages 118-141, MRID 46364801.
* Significantly different from control, $p \leq 0.05$.

2. **Body weight and food consumption**: Selected group mean body weight, body weight gain and food consumption values for pregnant or nursing dams are summarized in Table 4. Mean absolute body weight and food consumption were not affected by treatment during gestation. However, mean body weight gain in high-dose females was non-significantly decreased (90% of control value) for GDs 6-20. During lactation mean body weight for the high-dose dams was non-significantly decreased (94% of control value) on LD 4 and significantly decreased on LDs 7 through 21 (90-92% of control value). Body weight gain in high-dose females was decreased (45% of control value) during the lactation period. Food consumption in high-dose females was non-significantly decreased (92-94% of control value) during the second and third weeks of lactation.

TABLE 4. Selected mean (±SD) maternal body weight, body weight gain and food consumption[a]

Observations/study interval	Dietary concentration (ppm)			
	0	3	30	180
Gestation (n= 27-30)				
Body wt. Gestation day 6 (g)	224.2±2.9	230.6±2.9	231.8±2.6	225.8±3.5
Body wt. Gestation day 13 (g)	251.2±3.2	256.6±3.5	255.1±2.9	244.9±3.5
Body wt. Gestation day 20 (g)	310.8±4.3	319.2±4.8	316.2±4.1	303.7±4.3
Wt. gain gestation days 6-20 (g)	86.6±3.2	88.6±2.5	84.4±2.4	77.9±2.3 (90)
Food consumption gestation days 6-13 (g/day)	20.2±0.4	20.7±0.6	20.4±0.4	19.7±0.6
Food consumption gestation days 13-20 (g/day)	21.6±0.4	23.0±0.5	22.5±0.5	21.8±0.4
Lactation (n=20-30)				
Body wt. lactation day 0 (g)	239.6±3.3	245.3±3.6	246.5±2.9	234.8±3.8
Body wt. lactation day 4 (g)	256.6±3.8	257.6±4.4	262.7±3.1	241.7±5.3 (94)
Body wt. lactation day 7 (g)	266.0±4.0	270.2±4.9	270.1±3.0	243.3**±5.3 (91)
Body wt. lactation day 14 (g)	281.0±4.1	284.1±5.3	283.0±3.6	253.5**±5.8 (90)
Body wt. lactation day 21 (g)	271.6±3.7	277.3±4.3	278.0±3.4	249.3**±4.7 (92)
Wt gain lactation days 0-21(g)[b]	32.0	32.0	31.5	14.5 (45)
Food consumption lactation days 0-7 (g/day)	38.4±3.6	41.6±4.2	35.9±0.8	41.2±3.4
Food consumption lactation days 7-14 (g/day)	51.8±1.1	55.4±2.0	53.9±1.0	48.9±1.6 (94)
Food consumption lactation days 14-21 (g/day)	63.6±1.7	69.4±2.2	64.9±1.2	58.4±1.8 (92)

[a]Data obtained from pages 81, 83, 88 and 90, MRID 46364801.
[b]Calculated by the reviewer without standard deviations.
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer

3. **Test substance intake:** Based on maternal food consumption and body weight and nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 5.

TABLE 5. Mean maternal test substance intake (mg/kg body weight/day ± SE)[a]

Period	3 ppm	30 ppm	180 ppm
Gestation			
Days 6-13	0.3 ± 0.01	2.8 ± 0.06	16.5 ±0.60
Days 13-20	0.3 ± 0.01	2.8 ± 0.05	16.6 ± 0.23
Average	0.3	2.8	16.6
Lactation			
Days 0-7	0.6 ± 0.05	4.7 ± 0.12	33.5 ± 2.77
Days 7-14	0.7 ± 0.02	6.4 ± 0.09	38.3 ± 1.44
Days 14-21	0.8 ± 0.03	7.4 ± 0.11	42.7 ± 0.92
Average	0.7	6.2	38.2

[a]Data obtained from pages 45 and 92-93, MRID 46364801.

4. **Reproductive performance**: The fertility index was 93.3 for the control and high-dose groups, 100% for the low-dose group, and 90% for the mid-dose groups. The mean duration of gestation was comparable between control and treated groups. Results for the maternal animals are summarized in Table 6.

TABLE 6. Reproductive performance[a]

Observation	Dietary concentration (ppm)			
	0	3	30	180
Number mated	30	30	30	30
Number pregnant	28	30	27	28
Fertility index (%)	93.3	100.0	90.0	93.3
Intercurrent deaths	0	0	0	0
Mean (±SE) gestation duration (days)	21.7±0.1	21.7±0.1	21.8±0.1	21.5±0.1

[a] Data obtained from page 77, MRID 46364801.

5. **Cholinesterase activity**: Females exposed to 180 ppm had inhibition of plasma ChE (89%), RBC AChE (90%) and brain AChE (85%) activity on LD 21. Females at 30 ppm had inhibition of plasma ChE (77%), RBC AChE (85%) and brain AChE (49%) activity. Inhibition of plasma ChE (34%) and RBC (30%) AChE were observed at the 3 ppm dietary level. The data are included with offspring data in Table 15.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in the Table 7. The mean number of delivered pups per dam was not affected by treatment. The Live Birth Index and survival indices (Viability Index and Lactation Index) were unaffected by treatment. The number of offspring found dead or moribund after culling litters on PND 4 was 0, 1, 0 and 4 for the control, 3, 30 and 180 ppm groups, respectively. One pup at 3 ppm was found missing on PND 6 and pups at 180 ppm were found dead or missing on PND 7 and 9 (two females), 12 (one male) and 29 (one male). The cause of death for the high-dose pups that died during lactation was not established. All were gaining weight and had no clinical signs of toxicity prior to death.

   Sex ratio of pups was not reported. There were no treatment-related clinical signs of toxicity in offspring.

TABLE 7. Litter size and viability [a]

Observation	Dietary concentration (ppm)			
	0	3	30	180
Total number born	261	263	259	225
Pups/dam delivered	11.3±0.4	11.4±0.3	11.3±0.4	11.3±0.4
Number of litters	23	23	23	20
Number with live born litters	23	23	23	20
Litters with pups found dead	2	0	0	3
Number born live	261	262	258	225
Number born dead	0	1	1	0
Mean no. of viable pups				
Birth	11	11	11	11
Day 4 (precull)	11	11	11	11
Day 4 (postcull)	8	8	8	8
Day 21	8	8	8	8
Live Birth Index (±S.E.)	100.0 ± 0.0	99.6 ± 0.4	99.6 ± 0.4	100.0 ± 0.0
Viability Index (±S.E.)	98.2 ± 1.3	98.9 ± 0.6	100.0 ± 0.0	99.0 ± 0.7
Lactation Index (±S.E.)	100.0 ± 0.0	99.5 ± 0.5	100.0 ± 0.0	98.1 ± 1.0

[a] Data obtained from pages 95-96, MRID 46364801.

2. **Body weight:** Selected mean preweaning pup body weight and body weight gain data are presented in Table 8. Mean body weight of treated and control groups was similar at parturition but was significantly decreased in males (82-84% of control value) and females (81-85% of control value) at 180 ppm beginning on PND 11. Mean body weight gain was significantly decreased in males (79-85% of control value) and females (78-84% of control value) at 180 ppm throughout the study.

   Post-weaning (PNDs 28-70) body weight was significantly decreased in males (87-94% of control value) and females (89-94% of control value) at 180 ppm. Post-weaning body weight gain was unaffected by treatment. Selected mean post-weaning pup body weight and body weight gain data are presented in Table 9.

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TABLE 8. Selected mean (±SE) pre-weaning pup body weight and body weight gain [a]

PND	Dietary concentration (ppm)							
	0	3	30	180	0	3	30	180
	Males				Females			
Body Weight (g)								
0	5.9±0.1	5.9±0.1	6.2±0.1	5.8 ±0.1	5.5±0.1	5.6±0.1	5.9±0.1	5.5±0.1
4[b]	9.8±0.3	10.0±0.2	10.5±0.2	9.0±0.2 (92)	9.4±0.3	9.6±0.2	10.2±0.2	8.7±0.2 (93)
4[c]	9.8±0.3	10.0±0.2	10.5±0.3	9.1±0.2 (93)	9.4±0.3	9.6±0.2	10.1±0.2	8.8±0.2 (94)
11	24.9±0.5	25.4±0.8	26.4±0.5	20.9**±0.7 (84)	24.0±0.5	24.7±0.8	25.6±0.5	20.3**±0.6 (85)
17	38.7±0.7	38.6±1.0	39.6±0.6	31.8**±1.2 (82)	37.3±0.7	37.4±1.0	38.2±0.6	30.8**±1.1 (83)
21	48.9±1.0	49.5±1.1	50.4±0.9	39.9**±1.5 (82)	47.1±1.0	47.9±1.1	48.9±0.9	38.3**±1.4 (81)
Body Weight Gain (g)								
0-4	3.9±0.2	4.0±0.2	4.3±0.2	3.3*±0.2 (85)	3.8±0.2	4.0±0.2	4.3±0.2	3.2±0.2 (84)
4-11	15.1±0.3	15.4±0.6	15.9±0.4	11.9**±0.5 (79)	14.6±0.3	15.1±0.6	15.5±0.4	11.6**±0.5 (79)
4-21	39.1±0.8	39.5±0.9	39.8±0.8	30.9**±1.3 (79)	37.7±0.8	38.3±0.9	38.7±0.7	29.5**±1.2 (78)

PND = post-natal day
N=20-23
[a] Data obtained from pages 104-111, MRID 46364801.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically significantly different from control, p≤ 0.05
Number in parentheses is % of control value, calculated by reviewer.

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TABLE 9. Selected mean (±SD) post-weaning pup body weight and body weight gain [a]				
PND	Dietary concentration (ppm)			
	0	3	30	180
Males				
Body Weight (g)				
28	79.3±8.6	80.6±8.1	81.2±7.5	69.0*±10.7 (87)
35	125.3±12.1	127.5±11.3	129.1±10.6	112.6*±16.3 (90)
49	210.2±19.4	214.4±17.1	220.6*±18.5	197.2*±23.1 (94)
70	317.2±29.3	319.6±26.2	331.8*±26.2	295.4*±31.2 (93)
Body Weight Gain (g)[b]				
28-35	46.0	46.9	47.9	43.6
35-70	191.9	192.1	202.7	182.8
28-70	237.9	239.0	250.6	226.4
Females				
Body Weight (g)				
28	77.0±7.7	77.9±9.1	77.9±7.6	68.3*±9.4 (89)
35	114.3±9.4	114.7±10.9	115.3±9.3	104.6*±11.3 (92)
49	155.7±11.7	155.3±13.9	158.5±12.8	146.2*±14.8 (94)
70	198.0±16.9	198.3±17.0	199.6 ±16.7	185.2*±17.1 (94)
Body Weight Gain (g)[b]				
28-35	37.3	36.8	37.4	36.3
35-70	83.7	83.6	84.3	80.6
28-70	121.0	120.4	121.7	116.9

PND = post-natal day
[a] Data obtained from pages 115-116, MRID 46364801.
[b] Calculated by the reviewer without standard deviations.
* Statistically significantly different from control, p≤ 0.05
Number in parentheses is % of control value, calculated by reviewer.

3. Developmental landmarks:

a. Sexual maturation: The average age of onset of preputial separation in males was 44.0, 43.7, 42.7 and 44.6 days for the control, 3, 30 and 180 ppm groups, respectively. The average age of onset of vaginal opening was 33.3, 34.1, 33.9 and 33.4 days for the control, 3, 30 and 180 ppm groups, respectively. Body weight at attainment for males and females was not reported, although the protocol indicated that weights should be measured. Pupil constriction in response to a penlight was manifest in both control and treated groups by PND 21. (Pupil constriction was inadvertently not tested in pups necropsied on PND 21; this involved 18, 14, 12 and 10 pups in the control, 3, 30 and 180 ppm groups.) The data are presented in Table 10.

Table 10. Mean (±SE) age (days) at sexual maturation [a]

Parameter	Dietary concentration (ppm)			
	0	3	30	180
N (M/F)	23/23	23/23	23/23	20/20
Preputial separation mean age	44.0±0.4	43.7±0.5	42.7±0.3	44.6±0.8
Vaginal opening mean age	33.3±0.5	34.1±0.4	33.9±0.7	33.4±0.4
Pupil Constriction pups reaching criterion by PND 21 (%)	100	100	100	100

[a] Data obtained from page 113, MRID 46364801.

4. **Behavioral assessment:** In the following behavioral assessments, there were only 15 high-dose males at any testing period and 15 high-dose females after PND 4. The number of males was reduced to 14 on PND 60 after the death of one male on PND 29.

   a. **Functional observational battery**: No treatment-related FOB changes were observed at any of the testing periods (PNDs 4, 11, 21, 35, 45 and 60).

   b. **Motor/locomotor activity:** Total motor and locomotor activity data are presented in Table 11; individual motor activity data are shown in Appendix 1. Treatment-related increases in motor activity were noted in all male treatment groups on PND 17. Although the increases in mean levels of motor activity are not statistically significant, they are considered toxicologically significant and are evident in the individual animal data shown in the Appendix. The increases were most evident as a failure to habituate (i.e., there was little or no decrease in activity over the course of the test session). For example, in subsessions 4 and 5, control males had no counts over 50; 3 ppm males had 3 and 3 animals with counts over 50; 30 ppm males had 3 and 4 animals with counts over 50; and 180 ppm males had 5 and 2 animals with counts over 50, in subsessions 4 and 5, respectively. There was no treatment-related change in motor activity in females.

TABLE 11. Mean (±S.D.) motor and locomotor activity data (total activity counts for session) [a]								
PND	Dietary concentration (ppm)							
	0	3	30	180	0	3	30	180
	Males				Females			
Motor Activity								
13	49±44	66±50	78±80	79±64	58±60	81±95	44±36	75±82
17	127±85	199±108	169±107	220±142	153±100	208±146	158±91	227±145
21	331±77	310±66	314±106	358±124	346±126	412±226	296±84	325±100
60	529±157	501±103	519±98	552±107	645±179	614±144	662±174	666±190
Locomotor Activity								
13	4±5	2±2	6±8	5±5	3±2	6±9	4±4	4±8
17	27±27	46±25	45±38	57±44	33±22	58±46	39±35	58±42
21	94±24	96±29	103±38	107±48	97±32	116±67	88±29	91±33
60	365±135	330±89	364±80	393±97	435±146	391±125	444±161	456±168

PND = post-natal day
N=14-16
[a] Data obtained from pages 200-204, MRID 46364801.

c. **Auditory startle reflex habituation:** The summary amplitude and latency data for PNDs 22, 38±2 and 60±2 are presented in Tables 12. No treatment-related effects were observed. The startle response amplitude increased with age in both sexes, reflecting the age-related increase in the force of the response since body weight was subtracted from the response amplitude measure at each age. Habituation (decrease in response amplitude over the course of the test session) was apparent at all three ages.

TABLE 12. Auditory startle peak amplitude (g) and latency to peak (msec) (mean ±SD) [a]

Block	Parameter	0 ppm	3 ppm	30 ppm	180 ppm
Males - Postnatal Day 22					
1	Peak amplitude	51±19	45±25	53±17	41±16
	Latency to peak	34±5	38±8	37±7	42±8
2	Peak amplitude	47±23	41±22	46±23	37±17
	Latency to peak	34±5	39±10	34±6	37±10
3	Peak amplitude	45±18	42±26	45±21	38±21
	Latency to peak	34±4	35±6	32±6	37±8
4	Peak amplitude	42±17	37±23	41±20	37±19
	Latency to peak	34±6	37±8	34±6	36±10
5	Peak amplitude	40±14	36±22	38±15	31±14
	Latency to peak	33±4	37±7	34±6	37±9
mean	Peak amplitude	45±16	40±23	45±16	37±16
	Latency to peak	34±3	37±6	34±5	38±7
Body weight		68	80	76	71
Males - Postnatal Day 38					
1	Peak amplitude	84±36	104±83	88±47	91±34
	Latency to peak	35±4	33±5	35±5	35±4
2	Peak amplitude	78±43	85±63	76±37	81±40
	Latency to peak	33±6	32±5	33±4	35±5
3	Peak amplitude	68±39	75±55	70±51	77±32
	Latency to peak	34±6	34±5	34±5	36±6
4	Peak amplitude	63±37	62±44	57±38	63±23
	Latency to peak	35±5	35±6	36±6	36±6
5	Peak amplitude	65±43	58±38	64±58	60±25
	Latency to peak	35±5	36±5	34±5	35±5
mean	Peak amplitude	72±36	77±53	71±42	75±26
	Latency to peak	35±4	34±4	34±4	35±4
Body weight		140	139	149	128

TABLE 12. Auditory startle peak amplitude (g) and latency to peak (msec) (mean ±SD) [a]

Block	Parameter	0 ppm	3 ppm	30 ppm	180 ppm
Males - Postnatal Day 60					
1	Peak amplitude	212±121	238±169	233±145	231±165
	Latency to peak	37±3	38±3	38±4	38±4
2	Peak amplitude	222±157	205±168	211±178	202±155
	Latency to peak	34±3	37±4	36±5	37±5
3	Peak amplitude	142±67	155±120	141±136	172±127
	Latency to peak	35±4	37±6	37±6	36±4
4	Peak amplitude	120±70	130±99	128±90	135±65
	Latency to peak	37±4	35±6	38±6	37±5
5	Peak amplitude	106±58	113±66	112±78	115±61
	Latency to peak	37±5	37±4	37±5	37±3
mean	Peak amplitude	160±7	168±112	165±114	171±95
	Latency to peak	36±3	37±4	37±5	37±3
Body weight		273	274	291	265
Females - Postnatal Day 22					
1	Peak amplitude	42±14	48±16	53±19	46±21
	Latency to peak	38±7	37±7	36±8	37±8
2	Peak amplitude	43±14	42±16	48±21	43±18
	Latency to peak	35±6	40±10	34±4	37±6
3	Peak amplitude	36±14	38±15	45±23	41±19
	Latency to peak	37±7	39±10	33±6	36±8
4	Peak amplitude	33±14	37±14	41±21	38±18
	Latency to peak	37±6	36±9	32±4	35±7
5	Peak amplitude	36±16	35±16	38±16	35±17
	Latency to peak	36±6	36±7	34±8	36±6
mean	Peak amplitude	38±12	40±13	45±19	40±17
	Latency to peak	36±5	38±6	34±4	36±5
Body weight		69	58	52	43

TABLE 12. Auditory startle peak amplitude (g) and latency to peak (msec) (mean ±SD) [a]					
Block	**Parameter**	**0 ppm**	**3 ppm**	**30 ppm**	**180 ppm**
Females - Postnatal Day 38					
1	Peak amplitude	81±42	64±33	73±55	64±29
	Latency to peak	34±4	38±7	35±6	36±4
2	Peak amplitude	66±38	50±32	55±48	52±29
	Latency to peak	33±5	34±6	35±5	34±5
3	Peak amplitude	44±26	51±28	48±34	47±25
	Latency to peak	39±8	35±7	36±7	35±4
4	Peak amplitude	42±24	45±20	48±28	38±17
	Latency to peak	36±6	34±6	35±5	37±5
5	Peak amplitude	45±30	42±20	38±20	44±24
	Latency to peak	36±5	34±7	35±4	37±6
mean	Peak amplitude	56±29	50±22	52±35	49±23
	Latency to peak	35±4	35±5	35±4	36±4
Body weight		119	118	123	111
Females - Postnatal Day 60					
1	Peak amplitude	89±62	93±50	90±49	96±50
	Latency to peak	41±5	38±11	38±8	40±4
2	Peak amplitude	77±45	79±54	80±44	71±28
	Latency to peak	40±5	39±4	41±6	42±5
3	Peak amplitude	72±48	65±32	64±29	63±28
	Latency to peak	38±6	39±5	42±7	41±6
4	Peak amplitude	60±24	62±34	51±24	55±15
	Latency to peak	40±5	40±7	41±5	38±6
5	Peak amplitude	58±22	60±37	50±28	48±15
	Latency to peak	39±5	40±6	42±6	40±7
mean	Peak amplitude	71±34	70±37	69±36	67±22
	Latency to peak	40±3	37±10	39±7	40±4
Body weight		183	180	188	174

Data from pages 224-232
BW in grams; # of trials/block = 10; peak amplitude in grams; latency in milliseconds
N=16 for all groups except for 180 ppm males with N= 15

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d. Learning and memory testing:

1) Passive avoidance testing: The passive avoidance performance data for PNDs 22 and 29 are summarized in Table 13. No statistically significant differences occurred in the number of trials to criterion, trial latencies or number of rats that failed to learn.

TABLE 13. Passive avoidance performance data (mean ±SD) [a]

Parameter	Dietary concentration (ppm)			
	0	3	30	180
Males				
Session 1 (PND 22)[b]				
Number animals tested	16	16	16	15
Trials to criterion	3.0±0.4	3.0±0.0	2.9±0.3	2.9±0.4
Latency trial 1 (secs)	56.0±56.4	69.4±55.0	63.6±46.6	72.0±59.4
Latency trial 2 (secs)	173.1±27.5	180.0±0.0	180.0±0.0	180.0±0.0
No. failed to meet criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No. failed to cross during learning phase	1 (6%)	0 (0%)	1 (6%)	2 (13%)
Session 2 (PND 29)[b]				
Number of animals tested	15	16	15	13
Trials to criterion	2.1±0.5	2.3±0.7	2.1±0.4	2.3±0.6
Latency trial 1 (secs)	180.0±0.0	176.1±15.7	169.0±36.0	171.8±26.0
Latency trial 2 (secs)	173.6±24.7	175.9±13.0	180.0±0.0	76.3±13.5
Females				
Session 1 (PND 22)[b]				
Number animals tested	16	16	16	16
Trials to criterion	2.9±0.3	2.9±0.3	3.1±0.5	3.2±0.8
Latency trial 1 (secs)	58.4±61.5	48.7±45.7	46.0±31.3	48.9±49.8
Latency trial 2 (secs)	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0
No. failed to meet criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No. failed to cross during learning phase	2 (13%)	1 (6%)	0 (0%)	0 (0%)
Session 2 (PND 29)[b]				
Number of animals tested	14	15	16	16
Trials to criterion	2.2±0.6	2.0±0.0	2.1±0.5	2.1±0.3
Latency trial 1 (secs)	169.1±40.8	180.0±0.0	180.0±0.0	178.2±7.4
Latency trial 2 (secs)	174.7±19.8	180.0±0.0	177.2±11.2	180.0±0.0

[a] Data obtained from 234-235, MRID 46364801

[b] Session 1 = learning phase; Session 2 = retention phase

2) Watermaze performance: The watermaze performance data for PNDs 61±1 and 68±1 are summarized in Table 14. Treatment-related effects were noted in males at 180 ppm as a statistically significant increase in the number of trials to criterion and a non-statistically significant increase in the trial 2 duration in Session 1.

TABLE 14. Watermaze performance data (mean ±SD) [a]

Parameter	Dietary concentration (ppm)			
	0	3	30	180
Males				
Session 1 (PND 60±2)[b]				
Number of animals	16	16	16	15
Trials to criterion	6.7±1.4	7.1±2.5	5.9±1.5	8.5*±2.7
Trial 1 - errors	0.4±0.5	0.8±0.9	0.6±1.0	0.5±1.2
Trial 1 - duration (secs)	12.6±8.4	21.8±18.7	17.8±17.2	13.7±15.6
Trial 2 - errors	0.6±0.6	0.8±0.9	0.3±0.8	0.9±1.1
Trial 2 - duration (secs)	12.3±7.1	17.1±11.5	10.2±10.1	19.9±17.0
Failed to meet criterion	0 (0%)	1 (6%)	0 (0%)	1 (7%)
Session 2 (PND 67±2)[b]				
Number of animals	16	15	16	14
Trials to criterion	5.2±0.5	5.6±0.9	5.1±0.3	5.1±0.3
Trial 1 - errors	0.1±0.3	0.5±0.8	0.3±0.8	0.1±0.3
Trial 1 - duration (secs)	5.0±2.5	9.9±7.6	7.9±9.7	5.1±3.0
Trial 2 - errors	0.1±0.5	0.1±0.3	0.0±0.0	0.0±0.0
Trial 2 - duration (secs)	4.3±1.4	4.5±2.1	3.9±1.6	3.4±1.2
Females				
Session 1 (PND 60±2)[b]				
Number of animals	16	16	16	16
Trials to criterion	6.8±1.6	7.4±3.0	6.9±1.5	7.6±2.3
Trial 1 - errors	0.8±0.9	0.9±0.9	1.1±0.8	1.0±0.8
Trial 1 - duration (secs)	18.9±18.1	17.1±12.4	18.9±9.7	21.7±13.4
Trial 2 - errors	0.6±1.0	0.3±0.6	0.8±1.0	0.5±0.9
Trial 2 - duration (secs)	13.4±14.5	10.1±5.4	16.3±13.3	14.4*±18.1
Failed to meet criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (PND 67±2)[b]				
Number of animals	16	16	16	16
Trials to criterion	6.4±2.4	6.3±2.1	6.3±1.8	6.7±2.6
Trial 1 - errors	0.3±0.7	0.6±1.1	0.4±0.6	0.7±1.0
Trial 1 - duration (secs)	8.1±6.5	12.6±14.7	9.7±5.8	12.0±10.1
Trial 2 - errors	0.1±0.3	0.0±0.0	0.1±0.5	0.3±0.6
Trial 2 - duration (secs)	4.1±1.3	3.9±1.0	4.9±2.6	7.0±5.8

[a]Data obtained from 237-238, MRID 46342801

[b] Session 1 = learning phase; Session 2 = retention phase

5. **Cholinesterase activity:** Results of cholinesterase (ChE) activity assessment for dams and offspring are presented in Table 15. Pooled male and female offspring blood samples were tested on PND 4; the number of litters represented ranged from 13 to 20. On PND 21, the sample size was 6-10/sex/group, representing 16-20 litters. Ten dams/group were tested on LD 21.

In dams administered 180 ppm, inhibition of plasma ChE (89%), RBC AChE (90%) and brain AChE (85%) activities in relation to control values was measured on LD 21. At 30 ppm, inhibition of plasma ChE (77%), RBC AChE (85%) and brain AChE (49%) activities were reported. Inhibition of plasma ChE (34%) and RBC AChE (30%) activity occurred at 3 ppm.

On PND 4 in offspring, only plasma ChE (22%) activity was significantly inhibited at 180 ppm. On PND 21 in male and female offspring, plasma ChE (68-71%), RBC AChE (62-76%) and brain AChE (50-62%) ChE activities were significantly inhibited at 180 ppm. At 30 ppm, plasma ChE (32-40%) and brain AChE (7-10%) activities were significantly inhibited in both sexes.

TABLE 15. Cholinesterase activity in dams and offspring[a]

Cholinesterase activity (mean ± SD)	Dietary concentration (ppm)			
	0	3	30	180
Lactation day 21 dams- number	10	10	10	10
Plasma (IU/mL)	0.62±0.15	0.41$±0.05 (34)	0.14$±0.02 (77)	0.07$±0.02 (89)
RBC (IU/mL)	1.22±0.13	0.86$±0.17 (30)	0.18$±0.03 (85)	0.12$±0.02 (90)
Brain (IU/g)	11.3±0.7	11.5±0.5	5.8*±0.9 (49)	1.7*±0.5 (85)
Day 4 pooled male and female offspring - number	20	15	16	14/13[b]
Plasma (IU/mL)	0.58±0.12	0.51±0.05	0.52±0.05	0.45*±0.08 (22)
RBC (IU/mL)	1.24±0.27	1.29±0.19	1.28±0.27	1.09±0.32 (12)
Brain (IU/g)	3.7±0.5	3.5±0.4	3.8±0.3	3.5±0.4
Day 21 male offspring -number	10	10	10	10
Plasma (IU/mL)	0.56±0.06	0.54±0.08	0.38*±0.11 (32)	0.18*±0.06 (68)
RBC (IU/mL)	1.57±0.22	1.55±0.20	1.40±0.3 (11)	0.60*±0.44 (62)
Brain (IU/g)	10.1±0.5	10.5±0.7	9.1$±0.7 (10)	5.0$±2.1 (50)
Day 21 female offspring - number	10	10	8	6
Plasma (IU/mL)	0.55±0.07	0.52±0.09	0.33*±0.06 (40)	0.16*±0.08 (71)
RBC (IU/mL)	1.55±0.31	1.52±0.25	1.31±0.29 (15)	0.37*±0.34 (76)
Brain (IU/g)	10.2±0.2	9.9$±0.5 (3)	9.5$±0.6 (7)	3.9$±1.9 (62)

a Data obtained from pages 818-821, MRID 46364801.
b N = 14 for plasma and RBC, N = 13 for brain
* Statistically different from control, p<0.05, ANOVA + Dunnett's test.
$ Statistically different from control, p<0.05, Kruskal-Wallis ANOVA + Mann-Whitney U-test.
Number in parentheses is % inhibition relative to control from unnumbered table on page 53, MRID 46364801.

6. **Ophthalmology:** No treatment-related lesions were observed. Corneal opacity and retinal degeneration were observed but there was no dose-related increase in incidence.

7. Postmortem results:

a. **Brain weight:** Mean brain weight data are presented in Table 16. There were no treatment-related effects upon absolute mean brain weight in males and females.

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

Parameter	Dietary concentration (ppm)			
	0	3	30	180
Males				
Day 21 - Perfused				
Terminal body weight (g)	47.1±7.2	50.6±4.7	49.5±3.5	41.7±5.5 (89)
Fixed brain weight (g)	1.42±0.06	1.43±0.08	1.40±0.07	1.41±0.08
Day 75 - Perfused				
Terminal body weight (g)	322.1±29.7	325.9±25.3	345.1±23.9	289.5*±33.7 (90)
Fixed brain weight (g)	1.82±0.06	1.82±0.09	1.84±0.06	1.75±0.11
Day 75 - Non-perfused				
Terminal body weight (g)	318.9±25.5	322.3±26.4	336.5±21.7	313.9±23.4
Brain weight (g)	1.95±0.05	1.94±0.10	1.92±0.06	1.91±0.06
Females				
Day 21 - Perfused				
Terminal body weight (g)	47.2±4.6	48.6±3.6	48.1±5.1	36.5*±6.9 (77)
Fixed brain weight (g)	1.35±0.05	1.38±0.07	1.37±0.67	1.29±0.10
Day 75 - Perfused				
Terminal body weight (g)	200.9±15.8	194.1±16.7	199.7±21.1	186.9±14.4 (93)
Fixed brain weight (g)	1.69±0.06	1.73±0.10	1.71±0.07	1.68±0.09
Day 75 - Non-perfused				
Terminal body weight (g)	194.5±19.8	194.8±18.0	200.5±25.0	179.8±18.2 (92)
Brain weight (g)	1.72±0.09	1.82±0.10	1.76±0.12	1.71±0.09

[a] Data obtained from pages 857-864, MRID 46364801.
N=10
* Statistically different from control, $p<0.05$, ANOVA + Dunnett's test.
Number in parentheses is % of control value; calculated by reviewer.

b. **Macroscopic examination and measurement:** There were no findings related to treatment in animals that were found dead or sacrificed on PNDs 21and 75. At the PND 75 necropsy, one male from the 180-ppm group exhibited a dilated and perforated dorsal portion of the left side of the cerebrum. Microscopically, the lesion was considered a hydrocephalus due to the incomplete presence of brain tissue, dilatation and being partially lined only by the leptomeninges. This finding is not likely to be treatment-related; however, the study report should have cited the incidence of the finding in control Wistar rats from the conducting laboratory.

No treatment-related alterations were observed in the measurements of the cerebrum and cerebellum at the PND 21 or PND 75 necropsies. The data are presented in Table 17.

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TABLE 17. Mean (±SD) gross brain measurement data [a]

Parameter	Dietary concentration (ppm)			
	0	3	30	180
Males				
Day 21				
Cerebrum - anterior/posterior length (mm)	13.75±0.33	13.65±0.55	13.76±0.22	13.75±0.31
Cerebellum - anterior/posterior length (mm)	7.39±0.25	7.27±0.37	7.02±0.35	7.23±0.40
Day 75				
Cerebrum - anterior/posterior length (mm)	14.82±0.37	14.98±0.43	14.93±0.41	14.71±0.49
Cerebellum - anterior/posterior length (mm)	7.84±0.41	7.64±0.30	7.76±0.23	7.58±0.33
Females				
Day 21				
Cerebrum - anterior/posterior length (mm)	13.51±0.24	13.61±0.30	13.50±0.30	13.36±0.40
Cerebellum - anterior/posterior length (mm)	7.06±0.35	7.01±0.42	7.02±0.33	7.14±0.40
Day 75				
Cerebrum - anterior/posterior length (mm)	14.37±0.34	14.48±0.33	14.48±0.36	14.31±0.37
Cerebellum - anterior/posterior length (mm)	7.77±0.21	7.89±0.32	7.72±0.34	7.80±0.41

[a] Data obtained from pages 857-861, MRID 46364801.
N=10

c. **Neuropathology**:

1) **Microscopic examination:** As discussed above, one male at 180 ppm had hydrocephalus on microscopic examination at the terminal necropsy. This finding is not likely to be treatment-related; however, the study report should have cited the incidence of the finding in control Wistar rats from the conducting laboratory. Digestive chambers were present in a small number of sections of the cervical and lumbar dorsal root ganglion, tibial nerve, sciatic nerve and sural nerve in both control and high dose males and females. The incidences were not increased in other treated animals.

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2) **Brain Morphometry:** No treatment-related changes were observed in the results of six morphometric measurements taken from histologic sections. Data are presented in Table 18.

TABLE 18. Mean (±SD) microscopic brain measurement data [a]

Parameter	Dietary concentration (ppm)			
	Males		Females	
	0	180	0	180
Day 21				
Frontal Cortex	1.86±0.01	1.85±0.02	1.74±0.02	1.82±0.02
Parietal Cortex	2.00±0.01	1.95±0.00	1.95±0.01	1.86±0.04
Caudate Putamen	3.21±0.02	3.26±0.01	3.14±0.02	3.07±0.02
Hippocampus	1.72±0.00	1.70±0.00	1.58±0.01	1.57±0.01
Cerebellum	4.44±0.06	4.31±0.06	4.50±0.07	4.49±0.11
Day 75				
Frontal Cortex	1.75±0.01	1.68±0.02	1.76±0.01	1.78±0.01
Parietal Cortex	1.82±0.00	1.81±0.03	1.83±0.00	1.86±0.01
Caudate Putamen	3.40±0.05	3.37±0.05	3.36±0.03	3.36±0.02
Hippocampus	1.73±0.03	1.76±0.02	1.66±0.03	1.72±0.01
Cerebellum	4.30±0.04	4.24±0.09	4.42±0.05	4.45±0.11

[a] Data obtained from pages 866-874, MRID 46364801.
N=9-10

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study author established a parental LOAEL of 0.7 mg/kg/day based on RBC AChE inhibition on LD 21. The parental NOAEL was <0.7 mg/kg/day. The offspring LOAEL was 6.2 mg/kg/day based on brain AChE inhibition on PND 21. (NOAEL and LOAEL values were based on exposure during lactation since the effects were evident at the end of lactation.)

B. REVIEWER COMMENTS:

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

Doses during lactation were selected for the NOAEL and LOAEL, rather than lower doses during gestation, because effects were seen during lactation and not during gestation. It should be noted that several specifications of the DCI are not adequately addressed in the current protocol. The major inadequacy is a lack of information regarding duration of exposure and dose to the pups.

The registrant should address deficiency #3, below.

Historical control data on hydrocephaly should be submitted for complete documentation.

C. STUDY DEFICIENCIES:

1. The following comments from a January 25, 2001 EPA letter reviewing an Ethoprop developmental neurotoxicity study protocol were not adequately addressed in the study:

   a. Compound and important metabolites were not measured in the blood of dams and pups at the end of gestation, and in the milk during early, mid and late gestation. The EPA letter advised that if test substance was not present in the milk, direct exposure to the pups during lactation (e.g., gavage administration to the pups) might be needed in order to ensure adequacy of dosing.

   b. Pup food consumption (or sufficient data to permit a reliable estimate of pup intake) was not determined.

   c. Cholinesterase measurements were not taken in pups during mid-lactation to correlate with the same time points recommended for the milk measurements discussed above.

   d. Chemical-specific information was not submitted to justify the timing of the sample collection. No information was provided on the timing of cholinesterase activity measurements.

   e. Measurements of cholinesterase inhibition in a variety of peripheral nervous system tissues were not done, but were highly recommended.

   f. Individual measurements for both sides of the brain included in the morphometric analyses were not included in the study report.

2. Necropsy results on animals found dead were not included with the study report. Two males and one female at 180 ppm were found dead on PNDs 7, 12 and 29. According to the study methods, F1-generation animals were supposed to undergo a necropsy examination.

3. There is no explanation for the fewer number of females tested for ChE activity on PND 21. Ten males/sex/group and 10 females/sex in the control and 3 ppm groups were tested; however, only eight at 30 ppm and six at 180 ppm were evaluated. The same animals were used for ChE measurement, brain weight and gross brain measurements. Results of brain weight and gross measurements were reported for 10 pups/sex in all groups (Table OW1K, page 858).

APPENDIX 1: Individual motor activity data in males on postnatal day 17

Individual Motor Activity in Males PND 17						
Animal [a]	**Int. 1**	**Int. 2**	**Int. 3**	**Int. 4**	**Int. 5**	**Int. 6**
0 ppm						
1	22	6	69	0	2	9
2	15	21	37	38	0	0
3	25	5	50	0	2	0
4	80	47	23	0	41	44
5	52	20	0	10	5	0
6	1	0	0	0	23	20
7	112	29	12	10	0	26
8	63	17	4	0	0	0
9	61	0	11	12	0	0
10	43	26	68	46	0	0
11	67	33	0	0	0	0
12	3	0	0	0	0	0
13	28	0	0	0	0	0
14	75	0	96	18	7	5
15	148	90	25	0	47	27
16	74	46	30	0	0	0
Mean	54±40	21±24	27±30	8±14	8±15	8±14
3 ppm						
1	17	123	77	27	0	4
2	94	101	41	0	1	0
3	71	6	20	18	0	7
4	28	0	0	41	119	40
5	104	10	22	77	72	0
6	89	17	11	53	27	9
7	57	50	31	0	0	36
8	95	24	0	0	0	0
9	78	65	66	47	0	3
10	45	25	31	13	24	40
11	27	18	0	1	0	0
12	102	93	8	62	3	75

Individual Motor Activity in Males PND 17						
Animal [a]	Int. 1	Int. 2	Int. 3	Int. 4	Int. 5	Int. 6
13	32	23	0	0	0	0
14	124	81	54	30	93	71
15	49	7	42	0	0	0
16	69	7	38	12	0	1
Mean	68±32	41±40	28±24	24±25	21±38	18±260
30 ppm						
1	8	31	0	0	0	0
2	94	56	44	14	31	3
3	45	53	25	70	80	0
4	95	32	64	41	42	2
5	13	4	90	6	3	13
6	25	0	31	83	12	0
7	56	43	0	37	1	37
8	13	0	0	0	1	0
9	46	42	0	6	0	0
10	49	15	0	2	10	56
11	92	65	54	16	88	51
12	93	45	20	8	7	1
13	0	13	0	0	53	0
14	89	0	0	0	4	0
15	101	43	47	59	60	23
16	131	54	4	2	0	13
Mean	56±40	31±2	24±29	22±28	25±31	12±19

Individual Motor Activity in Males PND 17						
Animal [a]	Int. 1	Int. 2	Int. 3	Int. 4	Int. 5	Int. 6
180 ppm						
1	75	37	52	6	43	49
2	0	1	6	1	0	0
3	102	14	105	56	0	0
4	66	38	0	0	47	62
5	69	1	13	102	49	68
6	66	43	9	22	15	0
7	156	83	47	20	0	48
8	57	44	50	0	0	1
9	27	0	20	0	0	0
10	114	70	62	77	8	97
11	95	85	94	83	88	54
12	49	51	0	71	0	24
13	110	40	0	1	0	0
14	51	28	12	43	55	46
15	14	1	0	0	0	0
Mean	70±41	36±29	31±35	32±37	20±28	30±32

From pages 207 and 582-585

a Animal identified as 1-16 in this table, actual animal numbers in study are different.

APPENDIX 2: Preliminary Developmental Neurotoxicity Study

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

PC CODE: 041101 **TXR#**: 0052894
DP BARCODE: D309294 **SUBMISSION NO.**: N/A

TEST MATERIAL (PURITY): Ethoprophos (93.6% a.i.)

SYNONYMS: Ethoprop

CITATION: Sheets, L.P. (2004) Maternal and fetal cholinesterase activities in Wistar rats following dietary exposure during gestation to technical grade ethoprophos. Bayer CropScience LP Toxicology, Stilwell, KS. Study Number 02-D72-NN; September 10, 2004. MRID 46364802. Unpublished

EXECUTIVE SUMMARY: In a preliminary study intended to determine inhibition of ChE activity during gestation (MRID 46364802), Ethoprop (93.6 a.i., batch # OP 9950044) was administered to groups of 10-12 mated female Wistar Hannover Crl:WI (Glx/BRLHan) IGS BR rats in the diet at nominal concentrations of 0, 3, 30 or 240 ppm from gestation day (GD) 6 through GD 20 (inclusive). Mean intake based on average consumption during gestation weeks 2 and 3 was 0, 0.3, 2.5 and 21.4 mg/kg/day at 0, 3, 30 and 240 ppm, respectively. Maternal clinical signs, body weight and food consumption were assessed. Plasma ChE and RBC and whole-brain AChE activities were measured in the dam and fetus on GD 20.

No deaths or clinical signs of toxicity were observed in dams. Body weight in the high-dose group was significantly decreased (92% of control value) on GD 20 due to a significantly decreased (73% of control value) body weight gain during the treatment period. Food consumption was non-significantly decreased during GD 6-13 (88% of control value) and GD 13-20 (91% of control value).

In dams at 240 ppm, plasma, RBC and brain enzyme activities were inhibited, relative to control, 93%, 92% and 76%, respectively. At 30 ppm, plasma, RBC and brain enzyme activities were inhibited 87%, 77% and 10%, respectively. The only effect at 3 ppm was a 38% inhibition of plasma ChE activity in the dams.

In fetuses at 240 ppm, plasma ChE and RBC AChE activities were inhibited 20% and 34%, respectively. No treatment-related effects were observed at 3 or 30 ppm.

The maternal LOAEL for plasma ChE was 3 ppm. The maternal NOAEL for plasma ChE was not established.

The maternal LOAEL for RBC and brain AChE activity was 30 ppm. The maternal NOAEL for RBC and brain AChE activity was 3 ppm.

The fetal LOAEL was 240 ppm based on inhibition of plasma ChE and RBC AChE activity. The fetal NOAEL was 30 ppm.

This preliminary developmental neurotoxicity study in the rat is **Acceptable/Non-guideline**; it was conducted to determine dose levels for the definitive study.

13544

R144341

Chemical: Dichlorvos

PC Code: 084001

HED File Code:
Memo Date: 2/29/1996
File ID: 00000000
Accession #: 000-00-0120

HED Records Reference Center
4/23/2007