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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

July 19, 2005
TXR # 0052434

MEMORANDUM

Subject: 057801: Diazinon, Review of the Developmental Neurotoxicity Study

PC Code No.: 057801
DP Barcode No.: D299890

From: Marion Copley, DVM, DABT
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Through: Brenda May, Branch Senior Scientist
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I. CONCLUSIONS

The Developmental Neurotoxicity Study (MRID 46195601, 45842601) has been reviewed and is classified Aceptable /NonGuideline 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) due to the inadequacies in the assessment of motor activity in the offspring and the pending review of the positive control data. The attached review reflects the discussions/conclusions/classification by the DNT Work Group.

II. ACTION REQUESTED

HED was requested to review the Developmental Neurotoxicity Study (MRID 46195601, 45842601) on Diazinon.

JUL 2 2 2005

DATA EVALUATION RECORD

DIAZINON

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 46195601 (main study); 45822601 (range-finding)

Prepared for

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Disclaimer

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

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TXR#: 0052434

DATA EVALUATION RECORD

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

PC CODE: 057801 **DP BARCODE:** D299890

TEST MATERIAL (PURITY): Diazinon Technical (92.9%)

SYNONYMS: 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether

CITATION: Mandella, R.C. (2003) Diazinon: a developmental neurotoxicity study in rats. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study no. 01-4532; November 17, 2003. MRID 46195601. Unpublished.

Mandella, R.C. (2002) Diazinon: a dietary range-finding developmental neurotoxicity study in rats. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study no. 01-4530; November 13, 2002. MRID 45842601. Unpublished.

SPONSOR: Makhteshim-Agan of North America, 551 Fifth Avenue, Suite 1100, New York, NY 10176

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46195601), Diazinon technical (92.9% a.i., batch # 9896144) was administered to 27 female Crl:CD® (SD)BR IGS rats/dose in the diet at concentrations of 0, 0.30, 30 or 300 ppm from gestation day (GD) 6 through postnatal (lactation) day (PND) 21. The average daily test article intake was 0, 0.026, 2.36, or 24.2 mg/kg/day during gestation and 0.039, 4.06, or 39 mg/kg/day from GD 6 through PND 21. Dietary concentrations were based on a range-finding developmental neurotoxicity study in the rat (MRID 45842601). A Functional Operational Battery (FOB) was performed on 10 dams/dose on GDs 13 and 20, and on PNDss 7, 14, and 20. On PND day 4, litters were culled to yield five males and five females (as closely as possible). Offspring representing at least 20 litters/dose were allocated for detailed clinical observations (FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of auditory startle pre-pulse inhibition, assessment of learning and memory, and neuropathology at study termination (day 60 of age). On PND day 21, the whole brain was collected from 10

pups/sex/dietary level for micropathologic examination and morphometric analysis. Pup sexual maturation was assessed by age at vaginal opening for females and at completion of balano-preputial separation for males. Plasma, RBC, and brain cholinesterase activities were measured in dams on PND 21, and in selected pups on PNDs 4 and 21.

In dams, no treatment-related effects on mortality, clinical signs, body weight, FOB parameters, or necropsy findings were noted. Piloerection in a few high-dose animals (4/20) on one day (PND 20) is not clear evidence of maternal toxicity. There was a slight decrease in body weight gain (nonstatistical - 38%) and food consumption (10%) in the high dose dams during lactation. This is supported by the occurrence of cholinesterase inhibition in all three compartments at the mid dose, and to a greater extent the high dose (discussed below).

In mid- and high-dose dams, cholinesterase activity was significantly inhibited in all three compartments: enzyme activity was inhibited by 67 and 92.6%, respectively, in plasma, by 87.3 and 100%, respectively, in RBC, and by 24.8 and 86.8%, respectively, in brain. No inhibition was measured in low-dose dams.

For maternal systemic toxicity, the NOAEL is 30 ppm (2.36 mg/kg/day), the highest dose tested. A LOAEL was not established.

For maternal cholinesterase inhibition, the NOAEL is 0.3 ppm (0.026 mg/kg/day). The LOAEL is 30 ppm (2.36 mg/kg/day) based decreases in on plasma, RBC and brain cholinesterase activities.

Treatment had no adverse effects on survival, clinical sign, FOB, auditory startle response, brain weights, brain morphology or neuropathology.

No treatment-related effects were noted on body weight or body weight gain for pups in the low- or mid-dose groups. At the high dose, absolute body weights of male and female pups was significantly less ($p \leq 0.05$ or 0.01) than control pups beginning on PND 7 for males and PND 4 for females. Correspondingly, male and female pups from the high-dose group had significantly reduced body weight gain compared with the controls at all intervals throughout lactation. Post-weaning, offspring from the high-dose group had significantly lower body weight compared with the controls through PND 60 for males and PND 42 for females. Weight gain by the high-dose males was significantly less than that of the controls for the first two weeks, but was similar thereafter. Weight gain by the high-dose females was not affected during the post-weaning interval.

Males and females from the high-dose group had significant delays ($p \leq 0.01$) in preputial separation (1.9 days after control) or vaginal opening (1.3 days after control), respectively, compared with the controls. Mean body weight at attainment was similar between the treated and control groups for males and females.

In the assessment of motor activity, there was a possible treatment related effect in the high dose males on PND 17, however, it was difficult to interpret the biological significance of this since it only occurred on one day and there was large variability. It was also noted that activity for PND 21 was highly variable across intervals for both males and females. Overall, it was determined that the motor activity assessment was inadequate and difficult to interpret because of too much variability in the data.

In assessment of learning and memory, males from the high-dose group had significantly longer swimming time and a greater number of errors compared to the controls on the first and second day of testing at both PNDs 24 and 60.

In 4-day old male and female pups from high-dose litters, cholinesterase activity was significantly inhibited in plasma by 50.1 and 48.4%, respectively, in RBC by 41.3 and 37.8%, respectively, and in brain by 16.7 and 13.4%, respectively. On PND 21 dose-related inhibition of cholinesterase activity was observed in male and female pups from the mid- and high-dose litters. Plasma enzyme activity was inhibited in the mid- and high-dose offspring by 34.4 and 68.2%, respectively, in males and by 18.6 and 51.2%, respectively, in females. RBC enzyme activity was inhibited in mid- and high-dose males by 23.5 and 58.3%, respectively, and in high-dose females by 53.8%. Males and females from the high-dose group had 44.2 and 28.6% inhibition, respectively, of brain enzyme activity.

For offspring systemic toxicity, the NOAEL is 30 ppm (2.36 mg/kg/day). The LOAEL is 300 ppm (24.2 mg/kg/day) based reduced body weight and body weight gain an delayed sexual maturation in males and females.

For offspring cholinesterase inhibition NOAEL is 0.30 ppm (0.026 mg/kg/day). The LOAEL is 30 ppm (2.36 mg/kg/day) based on plasma, and RBC cholinesterase activities both sexes.

This study is classified **Acceptable/NonGuideline** 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) due to the inadequacies in the assessment of motor activity in the offspring and the pending review of the of 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: Diazinon Technical

Description:	yellowish liquid
Lot/Batch #:	9896144
Purity:	92.9 % a.i.
Compound Stability:	documented by sponsor; used before expiry date of 30 Oct. 2003
CAS # of TGAI:	333-41-5

2. Vehicle and/or positive control: The test article was stirred into acetone prior to adding to the diet.

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD® (SD)IGS BR	
Age at study initiation:	80-90 days	
Wt. at study initiation:	210-270 g at receipt (75-85 days)	
Source:	Charles River Laboratories, Kingston, NY	
Housing:	Individually or with litter in stainless steel grid or plastic cages	
Diet:	Certified Rodent Diet, No. 5002 (meal) from PMI Nurtition International, St. Louis, MO was available *ad libitum*.	
Water:	Tap water was available *ad libitum*.	
Environmental conditions:	**Temperature:**	20.5-25.8°C
	Humidity:	27.81-80.76%
	Air changes:	not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	4-6 days	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: January 26, 2003; End: April 24, 2003

2. Study schedule: Time-mated animals were received and assigned to study. The test substance was administered to the maternal animals from GD 6 through PND 21. A total of 27 dams/group was started on the study; on PND 6, 20 dams/group were randomly selected to continue on study and the excess dams and litters were sacrificed and discarded. Pups were weaned onto control diets on PND day 21, after which time maternal animals were killed. F_1 pups remained on study up to about PND day 60.

3. Mating procedure: Time-mated females were received from the supplier on GD 0, 1 or 2. No further details of the mating procedure were given. The day evidence of mating was found was designated GD 0 as determined at the supplier.

4. Animal assignment: Mated females were randomly allocated based on GD 0 body weight to the experimental groups shown in Table 1. Generally one male or one female offspring from

each litter (approximately 10 pups/sex/group) was allocated on PND 4 to each of the following: motor activity, auditory startle habituation, auditory startle inhibition, learning and memory, detailed observational battery, and sacrifice with cholinesterase determinations and brain weight on PND day 21. Cholinesterase activity in blood and brain was measured in randomly chosen culled pups on PND 4 and in selected pups on PND 21 (10/sex/group).

TABLE 1. Study design

Experimental parameter	Dietary concentration (ppm)			
	0	0.3	30	300
Maternal animals				
	No. of animals assigned			
FOB (GD 13, 20) (LD 7, 14, 20)	10 20-23	10 20-23	10 20-23	10 20-23
Blood cholinesterase determination (LD 21)	10	11	10	10
Brain cholinesterase determination (LD 21)	10	11	10	10
Offspring[1]				
Motor activity (PNDs 13, 17, 21, 60±2)	10/sex	10/sex	10/sex	10/sex
Detailed clinical/FOB (PNDs 4, 7, 11, 21, 35, 45, 60)	8-13/sex	8-13/sex	8-13/sex	8-13/sex
Auditory startle habituation (PNDs 23, 60±2)	10/sex	10/sex	10/sex	10/sex
Auditory startle pre-pulse inhibition (PNDs 23, 60±2)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PNDs 24, 62±2) (separate group used at each interval)	9-11/sex	9-11/sex	9-11/sex	9-11/sex
Gross necropsy and brain measurements (PND 21)	10/sex	10/sex	10/sex	10/sex
Neuropathology (PNDs 21, 60)	10/sex	10/sex	10/sex	10/sex
Blood cholinesterase determination (PNDs 4, 21)	10/sex	10/sex	10/sex	10/sex
Brain cholinesterase determination (PNDs 4, 21)	10/sex	10/sex	10/sex	10/sex

[1]one/litter/sex/dose when possible

5. **Dose selection rationale:** Dietary concentrations were chosen based on the results of a range-finding developmental neurotoxicity study in the rat (see Appendix; MRID 45842601), a two-generation reproductive toxicity study (MRID 41158101), and a range-finding developmental toxicity study. Briefly, at dietary concentrations of 300 and 500 ppm clinical signs of toxicity, decreased maternal weight gain, and decreased pup survival (500 ppm) were observed. NOAELs for cholinesterase inhibition in dams were 0.5 ppm for red cell and brain activity and 0.1 ppm for plasma activity. In offspring the NOAELs for cholinesterase inhibition in blood and brain were 10 ppm in fetuses and 50 ppm in pups. Therefore, dietary concentrations chosen for the current study were 0.3, 30, and 300 ppm.

6. **Dosage administration:** Diazinon was administered to female Sprague-Dawley rats in the diet at levels of 0, 0.3, 30 or 300 ppm from GD 6 through PND day 21. Offspring were potentially exposed in utero, through the milk, and directly late in lactation when they started eating the treated food.

7. **Dosage preparation and analysis:** Dietary formulations were prepared weekly. A premix for the high-dose group was prepared first. Approximately 2 kg of untreated diet was added to the bowl of a Hobart mixer. The appropriate amount of test article was added directly into a beaker containing 50 mL of acetone; the mixture was stirred using a stir bar. The acetone/test article mix was added to the bowl of the mixer and the beaker was rinsed twice with acetone and the washes added to the bowl. Using a paddle blade, the mixer was then run on speed 1 for approximately 15 minutes. Premixes for the low- and mid-dose groups were made by diluting an appropriate amount of the premix for the high-dose group with untreated food. Final diets were prepared by adding the premix to the required amount of food and mixing in a PK Twin-Shell Mixer for 15 minutes. The control diet was similarly prepared with 45 mL of acetone. Concentrations of the test substance in the diet were measured by GC with nitrogen phosphorus detection during the first, third, and sixth weeks of the study. Homogeneity and stability of the test article in the diet were determined in a previous study (HLS study no. 01-4530); these data were not included in the current report.

Results:

Homogeneity analysis: Homogeneity was noted as being confirmed in a previous report, however these data were not included in the current report.

Stability analysis: The test article was shown to be stable in the diet for 14 days under storage conditions used in the current study; data were not included.

Concentration analysis: Absence of test article was confirmed in the control diet. Mean concentrations of the low-, mid-, and high-dose test diets were 93.1, 90.3, and 91.0%, respectively, of nominal.

The report indicated that the concentration, stability, and homogeneity of the test article in the diets was adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** All animals were checked twice daily for general condition, mortality, or moribundity and once daily for clinical signs of toxicity. Each animal was subjected to a complete physical examination on GDs 0, 1 or 2 then on days 6, 7, 14, and 20 and on PNDs 1, 4, 7, 14, and 20.

Ten dams per group were observed (by observers blind to the treatment group) outside the home cage during the gestation dosing period (days 13 and 20) and 20-23 dams/group were observed during the lactation dosing period (days 7, 14, and 20). 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 extreme; 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., eye open, completely closed, slight drooping of eyelid, half closed.
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, 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.

There was no information regarding where the testing was done (other than to say in an open arena) and what the environmental conditions (e.g., noise level, etc.) were.

Individual maternal body weight was recorded on GDs 0 (from supplier), 3, 6, 10, 14, 17, and 20, on the day of parturition, and on PNDs 1, 4, 7, 11, 14, 17, and 21. Food consumption was measured on GDs 3-6, 6-10, 10-14, 14-17, and 17-20, and on PNDs 1-4, 4-7, 7-11, 11-14, 14-17, and 17-21. Food consumption measurements may have included consumption by the pups, especially during lactation week 3.

From GD 18, dams were checked twice daily for evidence of parturition. They were permitted to deliver and rear offspring until PND day 21. Numbers of live and dead offspring were recorded during parturition.

b. Offspring:

1) **Litter observations:** Litters were examined as soon as possible after birth for the number of live and dead pups, pup abnormalities, and the sex of each pup. Litters were examined twice daily throughout lactation for mortality and once daily for clinical signs of toxicity.

   On day 4 postpartum, litters were standardized to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible); excess pups were killed and discarded. Litters with less than 8 pups were excluded from further testing.

2) **Developmental landmarks:** Pups were sexed after parturition (PND 0) and the sex verified on PNDs 4, 11, 17 and 21.All pups were observed for eye opening beginning on PND 13 (bilateral opening needed to consider opening complete). Beginning on PND day 37, male offspring were examined daily for balanopreputial separation. Beginning on PND day 28, female offspring were examined daily for vaginal patency. The body weight at attainment of sexual maturation was recorded.

3) **Detailed observations:** Offspring were examined for clinical signs once daily. Each animal was subjected to a full physical examination on PNDs 0, 4, 7, 11, 14, 17, 21, 28, 35, 45, 52, and 60. Individual offspring body weight data were recorded on PNDs 1, 4, 7, 11, 17, and 21, and once weekly thereafter. Food consumption by the offspring was not measured.

4) **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report (also see Table 1) .

i. **Functional observational battery (FOB):** On PND days 4, 7, 11, 21, 35, 45, and 60, 8 - 13 offspring/sex/group were examined outside the home cage in an FOB assessment by observers blind to the treatment groups. On PND days 7 and 11, the animals were tested for surface righting reflex (score: 1 = immediately or up to 2 sec; 2 = slow, 3-5 sec; 3 = fail, >5 sec). The duration of the open field observation was not given for each age.

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., eye open, completely closed, slight drooping of eyelid, half closed.
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, 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.

ii. **Motor activity testing:** Motor activity was evaluated in 10 pups/sex/group on days 13, 17, 21, and 60±2. Activity was monitored using an automated Photobeam Activity System (PAS; San Diego Instruments, Inc.) during a 60-minute session composed of twelve 5-minute intervals. The total number of photobeam breaks was recorded for each 5-minute interval.

iii. **Auditory startle habituation and pre-pulse inhibition:** These tests were performed on a minimum of 10 offspring/sex/dose on PNDs 23 and 60±2, using a San Diego Instruments SRS System. "A separate subset of animals ... was used for habituation and for pre-pulse inhibition. Animals were acclimated to the auditory startle chamber for 5 minutes prior to the start of the evaluations."

AUDITORY STARTLE HABITUATION: After acclimation the animals were presented with the startle stimulus at 15-second intervals during 5 consecutive blocks of 10 trials. The startle stimulus consisted of 50 millisecond bursts of white noise at 111.8±3.1 decibels (dB) against a background noise level of 68.2±3.8 dB.

PRE-PULSE INHIBITION OF STARTLE: Responses were recorded for 10 trials with a pre-pulse of sound immediately preceding the startle stimulus and for 10 trials without a pre-pulse. The 20 trials were presented in semi-random order. The startle stimulus was not defined and is assumed to be the same as that used in the habituation trials. The pre-pulse stimulus was 72.05±1.7 dB.

iv. **Learning and memory testing:** Learning and memory testing was performed on 9-11 offspring/sex/dose on PNDs 24 and 62±2 using a Biel water maze (multiple-T maze). Different pups were used at each interval. The report stated: "The maze was filled with water at room temperature. Each rat was placed in the maze for a maximum time of 3 minutes per testing event. Each testing event was considered a trial and there was a minimum of 2 hours between trials. Each rat ... had 2 trials per day of testing. On the initial day of testing, each pup was placed in the maze and allowed to swim in a straight line to the exit ramp" (4 feet) and the time recorded. "The learning component of this test was conducted on 4 subsequent days at which time the rat was required to swim the entire maze. The number of errors and the time to complete the maze was recorded. After a 2-day rest period each rat was required to swim the entire maze for evaluation of memory."

5) **Opthalomology:** Ophthalmoscopic examinations were not done.

6) **Cholinesterase determination:** Plasma, erythrocyte, and brain cholinesterase activities were measured in randomly chosen culled pups on PND 4 and in selected pups and dams on PND 21 (10/sex/group). PND 4 pups were anesthetized with CO_2 and the heart and great vessels exposed; vessels anterior to the heart were severed and blood was collected via heparinized syringes or microcapillary tubes. PND 21 pups and dams were anesthetized with CO_2 or CO_2/O_2, respectively, and blood was collected from the vena cava via heparinized syringes or tubes. All blood samples were centrifuged to obtain plasma and erythrocytes and each fraction was frozen (-70°C) until analysis. After blood collection, animals were sacrificed by exsanguination and the brain removed, weighed, and homogenized. The brain homogenate was stored frozen until analysis.

Cholinesterase assays were conducted using a modified Ellman technique. Assays used an optimal wavelength of 412±10 nm, a phosphate (working) assay buffer with an optimal pH of 8.0, an optimal final substrate concentration in the reaction mixture for rat tissues of 1.0 mM, a final concentration of the color indicator (DTNB) of not less than 0.25 mM, and a reaction temperature of 37°C. Both substrate and tissue blanks were run with each assay.

2. **Postmortem observations:**

a. **Maternal animals:** Excess females with litters not needed for further evaluation were euthanized on PND 6 and discarded. Maternal animals not used for cholinesterase

determination were sacrificed by carbon dioxide inhalation on PND 21 and subjected to gross necropsy. Any abnormal tissues were preserved in 10% neutral buffered formalin.

b. **Offspring:** Dead pups or those sacrificed moribund were subjected to gross necropsy. On PND 4 culled pups were sacrificed by an intraperitoneal injection of sodium pentobarbital and examined externally. Offspring were sacrificed and examined on PNDs 21 or approximately 60. Those animals not allocated for neuropathological examination or cholinesterase measurement were killed by carbon dioxide inhalation, examined grossly, and the brain removed and weighed. The offspring selected for neuropathology/morphometry evaluation were subjected to postmortem examinations as described below.

At PND day 21, up to 10 pups/sex/group were sacrificed by intraperitoneal injection of sodium pentobarbital and perfused via the left ventricle with phosphate-buffered saline followed by 1% gluteraldehyde/4% paraformaldehyde in buffer. Following perfusion, the animals were refrigerated for approximately 1 hour. The brain was collected and preserved in fixative. Brains from all dose groups were embedded in paraffin and were sectioned for control and high-dose animals. Tissues were sectioned at 4-5 μm and stained with hematoxylin and eosin. Sections of cerebral cortex, olfactory bulbs, hippocampus, basal ganglia, mid-brain (tectum, tegmentum, and cerebral peduncles), brain stem, cerebellum, thalamus, and hypothalamus from control and high-dose animals were examined microscopically.

The following brain morphometric measurements were performed:

Neocortex thickness (distance from the pial surface to the top of the white matter along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits its greatest thickness)

Hippocampus thickness (greatest dorsal-ventral thickness)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between its tip and base)

On PND day 60, 10 rats/sex/dose were were sacrificed by intraperitoneal injection of sodium pentobarbital and perfused via the left ventricle with phosphate-buffered saline followed by 1% gluteraldehyde/4% paraformaldehyde in buffer. Following perfusion, the animals were refrigerated for approximately 1 hour. The brain, spinal cord, both eyes with optic nerves, peripheral nerves (tibial and sciatic), dorsal root fibers and ganglia, ventral root fibers, and gastrocnemius muscle were collected and preserved in fixative.

The following central and peripheral nervous system tissues were dissected and embedded in paraffin (CNS tissues) or plastic (PNS tissues): cerebral cortex, olfactory bulbs, hippocampus, basal ganglia, mid-brain (tectum, tegmentum, and cerebral

peduncles), brain stem, cerebellum, thalamus, hypothalamus, spinal cord, dorsal root ganglia, dorsal root fibers, ventral root fibers, eyes, optic nerve, skeletal muscle, sciatic nerve, and tibial nerve. 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 4-5 µm and stained with hematoxylin and eosin. Plastic-embedded tissues were sectioned at 2 µm and stained with toluidine blue.

The following brain morphometric measurements were performed:

Neocortex thickness (distance from the pial surface to the top of the white matter along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits its greatest thickness)

Hippocampus thickness (greatest dorsal-ventral thickness)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between its tip and base)

D. DATA ANALYSIS:

1. **Statistical analyses:** Body weight, body weight gain, food consumption, gestation length, number of pups/litter, and learning and memory data were analyzed for equality of means by a one-way analysis of variance (ANOVA) using the F ratio to assess significance. Developmental landmark data were analyzed using a one-way analysis of covariance (ANCOVA) with body weight as the covariant. If significance was indicated, the ANOVA or ANCOVA was followed by Dunnett's test. Offspring survival indices were analyzed by Fisher Exact Test with Bonferroni correction. Brain weight, morphometry, cholinesterase, auditory startle, and surface righting data were analyzed for homogeneity of variance using Bartlett's test. If Bartlett's was not significant, the ANOVA followed by Dunnett's test for non-monotonic responses or Williams test for monotonic responses was used. If Bartlett's was significant, Cochran and Cox's modified t-test (brain weight data) was used or a Kruskal-Wallis test was used followed by Steel's test or Shirley's test. Motor activity and auditory startle data were analyzed using split-plot repeated measures ANOVA with analyses for group by interval interactions and overall treatment effects; if significance was found, Dunnett's test was used to compare the treated groups with the control group.

   These statistical analyses appear appropriate.

2. **Indices:**

   a. **Reproductive indices:** A gestation index was listed in one of the results tables but the formula used for calculating it was not given.

   b. **Offspring viability indices:** Live birth and viability indices were listed in one of the results tables but the formula used in calculation was not given.

3. **Positive and historical control data:** Actual positive and historical control data are not presented and no references to such data were given in the report. HED has reviewed the positive control data that was submitted by this testing facility for another chemical. It was concluded that, "These data do not support proficiency." The data were inadequate as provided. The year of the study was not present. Data were provided for treatment groups only, with no control for comparison, no dose/response information, no procedural information (data consisted of tables of results only), including no information on treatment parameters (e.g. time and methods of dosing, duration of dosing, etc.) and there were only summary tables. "If more complete information were provided, including data for control groups, it is possible that positive control data for the provided endpoints might be satisfied. No data were provided for several endpoints, including histopathology, morphometrics, and FOB, therefore gaps would remain even if studies for available endpoints were satisfactory. We note that historical control data were provided for motor activity in day 17 females (graph only, presenting data for 4 separate control groups)."

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No dams were found dead or were sacrificed in moribund condition during gestation or lactation. No treatment-related clinical signs of toxicity were observed during gestation at either cage-side observation or during the FOB. On PND 20, 4/20 high-dose dams were observed with piloerection compared with 1-2 animals in the other control and treated groups. Hair loss was a common finding on animals in the treated and control groups.

2. **Body weight and food consumption:** Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 2. There were no significant treatment-related effects on body weight, or body weight gain, or food consumption observed during gestation. Food consumption by the treated groups was occasionally greater than that of the control group during gestation but this was not considered treatment related. There were no statistically significant treatment-related effects on body weight, or body weight gain during lactation. However, there was a non-statistical decrease in body weight gain (about 37%) during most of lactation. This is consistent with the slight decrease observed in the range-finding study. In addition, food consumption in the high-dose group was significantly less (about 10%) than that of the controls during PNDs 14-21, indicating a possible treatment related effect.

TABLE 2. Selected mean (±SD) maternal body weight and food consumption				
Observation/study interval	0 ppm	0.3 ppm	30 ppm	300 ppm
Gestation (n=)	(27)	(24)	(26-27)	(27)
Body wt. GD 0 (g)	230 ± 11.2	229 ± 11.3	230 ± 11.4	231 ± 12.9
Body wt. GD 6 (g)	265 ± 13.0	266 ± 13.1	265 ± 12.9	267 ± 14.3
Body wt. GD 14 (g)	320 ± 15.8	324 ± 19.5	320 ± 19.5	319 ± 16.4
Body wt. GD 20 (g)	393 ± 21.1	400 ± 28.0	394 ± 23.6	396 ± 21.6
Wt. gain GDs 6-10	25 ± 7.9	27 ± 7.0	25 ± 8.2	21 ± 7.8
Wt. gain GDs 0-20 (g)	162 ± 16.8	171 ± 23.1	164 ± 20.6	165 ± 16.9
Food consumption GDs 6-10 (g/rat/day)	23 ± 1.6	25 ± 2.3	23 ± 4.0	23 ± 2.1
Food consumption GDs 17-20 (g/rat/day)	24 ± 1.8	26 ± 3.1	25 ± 2.6	26* ± 2.1
Lactation (n=17-27)[a]				
Body wt. PND 0 (g)	297 ± 20.9	304 ± 21.7	299 ± 18.5	300 ± 19.6
Body wt. PND 4 (g)	311 ± 20.0	319 ± 25.8	312 ± 19.2	307 ± 18.6
Body wt. PND 14 (g)	339 ± 15.3	344 ± 23.2	340 ± 22.0	324 ± 21.4
Body wt. PND 21(g)	332 ± 19.0	334 ± 21.1	328 ± 23.1	322 ± 17.2
Wt. gain PNDs 1-4 (g)	13 ± 12.1	14 ± 9.4	13 ± 10.7	9 ± 7.0
Wt. gain PNDs 1-21 (g)	37 ± 16.5	31 ± 14.8	30 ± 16.9	23 ± 19.4
Food consumption PND 1-4 (g/day)	35 ± 10.0	35 ± 4.8	34 ± 5.3	35 ± 3.6
Food consumption PND 17-21 (g/day)	76 ± 6.9	72 ± 7.1	75 ± 7.3	68** ± 8.9

Data obtained from Tables 4-6, pp. 87-89, and Tables 10-12, pp. 98-100, MRID 46195601.
[a]Dam not weighed on PND 0 if delivery was in progress; excess dams and litters (>required 20/group) were sacrificed after day 6.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

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 3.

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day)			
Period	0.3 ppm	30 ppm	300 ppm
GDs 6-20	0.026	2.36	24.2
PNDs 1-14	0.043	4.60	44.2
PNDs 14-21	0.060	6.43	59.0
Overall average dose	0.039	4.06	39.0

Data obtained from Text Table 2, p. 47, MRID 46195601.

4. **Reproductive performance:** Reproductive performance is summarized in Table 4. Gestation length, number of live litters born, and gestation index were similar between the treated and control groups.

TABLE 4. Reproductive performance				
Observation	**0 ppm**	**0.3 ppm**	**30 ppm**	**300 ppm**
Number assigned (pregnant)	27 (27)	27 (24)	27 (27)	27 (27)
Gestation length (days)	21.7 ± 0.54	21.5 ± 0.51	21.6 ± 0.49	21.5 ± 0.51
Number of live litters born	27	24	27	27
Gestation index (%)	100	100	100	100

Data obtained from Table 14, p. 103, MRID 46195601.

5. **Maternal postmortem results:** No treatment-related abnormalities were found at maternal necropsy.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results of pups during lactation are summarized in Table 5. No treatment-related effect on the number of litters, live litter size, sex ratio, or live birth or viability indices was observed. One low-dose dam had total litter loss by day 4. This litter was not used in the calculations for litter size or survival but should have been included; this omission does not compromise the integrity of the study.

   No treatment-related clinical signs of toxicity were observed in the offspring during lactation or during the post-weaning period.

TABLE 5. Litter size and viability				
Observation	**0 ppm**	**0.3 ppm**	**30 ppm**	**300 ppm**
Number of live litters	27	24	27	27
Total litter loss (by PND 4)	0	1	0	0
Total stillborn pups(litters involved)	0(0)	2(2)	1(1)	3(3)
Mean no. of viable pups[a]				
Day 1	13.0 ± 2.59	12.7 ± 2.53	13.3 ± 1.85	12.9 ± 1.96
Day 4 (precull)	12.8 ± 2.61	12.5 ± 2.59	13.1 ± 1.83	12.8 ± 1.89
Day 4 (postcull)	9.6 ± 1.28	9.8 ± 0.60	10.0 ± 0.0	9.9 ± 0.42
Day 21	9.9 ± 0.31	9.8 ± 0.51	10.0 ± 0.0	9.8 ± 0.55
Post-implantation survival index (%)[a]	93.9	95.7	92.2	92.4
Live birth index (%)	100	99.4	99.7	99.2
Viability index (%)	98.3	97.6	98.9	95.0
Sex ratio day 0 (%male)	52.1	50.5	50.7	47.4

Data obtained from Table 14, pp. 104-106, respectively, MRID 46195601.
[a]Based on 20-21 litters/group after day 6.

2. **Body weight:** Selected offspring body weight data are given in Table 6 for the lactation interval. Offspring body weight was similar between the treated and control groups on PND 1. However, absolute body weight of male and female pups from high-dose litters was significantly less than that of the control pups beginning on PND 7 for males and PND 4 for

females. Correspondingly, male and female pups from the high-dose group had significantly reduced body weight gain compared with the controls at all intervals throughout lactation.

Post-weaning, offspring from the high-dose group had significantly lower body weight compared with the controls through day 60 for males and day 42 for females (Table 7). Weight gain by the high-dose males was significantly less than that of the control for the first two weeks, but was similar thereafter. Weight gain by the high-dose females was not affected during the post-weaning interval. No treatment-related effects were noted on body weight or body weight gain for pups in the low- or mid-dose groups.

TABLE 6. Mean (±SD) pre-weaning pup body weight and body weight gain (g)

Day of age/ interval	0 ppm	0.3 ppm	30 ppm	300 ppm
Males				
1	7.6 ± 0.94	7.5 ± 0.83	7.6 ± 0.67	7.4 ± 0.68
4 (post-cull)	11.0 ± 1.63	11.0 ± 1.43	10.9 ± 1.08	10.3 ± 1.18
7	16.9 ± 2.18	16.9 ± 1.86	16.8 ± 1.32	15.4* ± 1.84 (91)[a]
11	24.5 ± 2.07	24.5 ± 2.00	25.2 ± 1.63	22.4** ± 2.93 (91)
17	36.3 ± 3.21	36.0 ± 3.47	37.2 ± 2.51	30.3** ± 4.00 (83)
21	49.4 ± 4.10	48.2 ± 4.32	50.1 ± 3.35	40.5** ± 6.42 (82)
Wt. gain days 1-4	3.5 ± 0.76	3.5 ± 0.69	3.3 ± 0.62	2.9* ± 0.75 (83)
Wt. gain days 4-21	38.9 ± 3.31	37.3 ± 3.66	39.4 ± 2.77	30.3** ± 5.67 (78)
Females				
1	7.2 ± 0.90	7.0 ± 0.82	7.2 ± 0.64	7.0 ± 0.70
4 (post-cull)	10.7 ± 1.54	10.5 ± 1.31	10.4 ± 1.01	9.7* ± 1.22
7	16.5 ± 2.06	16.0 ± 1.76	16.0 ± 1.23	14.4** ± 1.86 (87)
11	24.0 ± 2.03	23.5 ± 2.13	24.4 ± 1.22	21.0** ± 2.6 (87)
17	35.3 ± 3.10	34.6 ± 3.53	36.1 ± 2.21	29.3** ± 3.28 (82)
21	48.2 ± 3.73	46.4 ± 4.60	48.1 ± 2.79	39.2** ± 5.39 (81)
Wt. gain days 1-4	3.4 ± 0.77	3.4 ± 0.66	3.2 ± 0.59	2.8** ± 0.76 (82)
Wt. gain days 4-21	38.0 ± 3.00	36.1 ± 3.84	37.9 ± 2.25	29.6** ± 4.59 (78)

Data obtained from Tables 19 and 20, pp. 132-134 and 137-138, respectively, MRID 46195601.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.
[a]Number in parentheses is percent of control; calculated by reviewer.

TABLE 7. Mean (±SD) post-weaning offspring body weight and body weight gain (g)				
Day of age	0 ppm	0.3 ppm	30 ppm	300 ppm
Males				
28	90 ± 7.7	89 ± 8.3	93 ± 5.9	78** ± 11.8 (87)[a]
35	153 ± 12.0	154 ± 12.6	161** ± 10.1	136** ± 19.2 (89)
42	217 ± 15.6	219 ± 16.0	227** ± 14.9	197** ± 23.2 (91)
49	280 ± 21.4	280 ± 20.4	292** ± 19.7	258** ± 27.4 (92)
56	340 ± 26.4	342 ± 25.4	358** ± 25.8	319** ± 32.3 (94)
60	372 ± 24.7	362 ± 27.7	385 ± 28.1	348** ± 36.4 (94)
Wt. gain days 21-28	41 ± 4.4	41 ± 4.6	43* ± 4.5	38** ± 6.2 (93)
Wt. gain days 35-42	63 ± 5.6	65 ± 6.2	66 ± 6.7	61 ± 6.1
Wt. gain days 56-60	28 ± 5.5	25* ± 6.6	28 ± 6.8	28 ± 5.6
Females				
28	83 ± 6.8	82 ± 7.5	84 ± 6.0	73** ± 11.0 (88)
35	131 ± 9.2	131 ± 9.6	130 ± 8.1	120** ± 13.9 (92)
42	164 ± 10.7	168 ± 12.0	166 ± 8.7	159* ± 16.4 (97)
49	191 ± 13.2	197 ± 14.0	194 ± 11.6	187 ± 18.1
56	216 ± 15.9	222 ± 17.9	217 ± 15.2	214 ± 20.5
60	227 ± 15.1	233 ± 21.0	229 ± 19.1	224 ± 18.8
Wt. gain days 21-28	35 ± 4.4	35 ± 4.1	35 ± 4.1	34 ± 4.9
Wt. gain days 35-42	33 ± 5.2	37** ± 6.5	35 ± 4.2	38** ± 7.8
Wt. gain days 56-60	9 ± 5.6	11 ± 5.3	11 ± 5.4	10 ± 4.4

Data obtained from Tables 19 and 20, pp. 135-136 and 141-142, respectively. MRID 46195601.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

3. Developmental landmarks:

a. **Sexual maturation:** Age and body weight at sexual maturation is given in Table 8. Males and females from the high-dose group had significant delays in preputial separation or vaginal opening, respectively, compared with the controls. However, mean body weight at attainment was similar between the treated and control groups for males and females. Therefore, the delays are attributable to body weight, associated with delayed growth rather than a direct delay in development.

TABLE 8. Mean (±SD) age and body weight at sexual maturation				
Parameter	0 ppm	0.3 ppm	30 ppm	300 ppm
N (M/F)	63/60	64/66	60/60	59/60
Males				
Preputial separation (days)	43.1 ± 1.41	43.9 ± 1.32	43.6 ± 1.28	45.0** ± 1.88
Body wt. at attainment (g)	225 ± 18.6	232 ± 17.3	241** ± 17.6	222 ± 19.0
Females				
Vaginal opening (days)	32.5 ± 1.10	33.1 ± 1.69	32.6 ± 1.31	33.8** ± 2.28
Body wt. at attainment (g)	114 ± 9.5	116 ± 13.4	113 ± 10.7	110 ± 16.6

Data obtained from Table 18, pp. 130-131, MRID 46195601.
Significantly different from control: **p ≤ 0.01.

b. **Developmental landmarks:** The mean time to eye opening was 14.4-14.8 days for all groups. Other developmental landmarks, such as incisor eruption, pinna unfolding, and fur growth, were not monitored.

4. **Behavioral assessments:**

a. **Functional observational battery:** No treatment-related effects were found during in-hand or arena observations on offspring on any test day (PND 4, 7, 11, 21, 35, 45, or 60). Mean scores for surface righting reflex were similar between the treated and control groups on PND 7 and 11.

b. **Motor/locomotor activity:** Mean total activity counts are given in Table 9, Subsessions for the males are in Tables 10. Motor and locomotor activities were not presented separately and the overall mean for the session was not calculated. Therefore, the reviewer calculated the session mean for each testing day from the mean interval data given in the report. No treatment-related effects were observed on any testing day. Significantly greater activity than that of the controls was observed for males in the high-dose group on PND 17 and for males in the mid-dose group on PND 13. For the treated groups, activity for a few intervals was greater or less than the control group, but no consistent dose- or time-related pattern was observed. Habituation was evident in most groups by PND 17. Although there is a possible treatment related effect in the high dose males on PND 17, it is difficult to interpret the biological significance of this since it only occurred on one day and there was large variability. It was also noted that activity for PND 21 was highly variable across intervals for both males and females. Overall, it was determined that the motor activity assessment was inadequate and difficult to interpret because of too much variability in the data.

TABLE 9. Mean (±S.D.) motor activity data[a]

Test day	0 ppm	0.3 ppm	30 ppm	300 ppm
Males				
PND 13	5.6 ± 3.9	14.1 ± 7.0	20.3* ± 8.9	7.6 ± 3.6
PND 17	13.3 ± 10.0	19.9 ± 7.2	23.9 ± 18.1	42.8* ± 11.9
PND 21	21.2 ± 15.2	21.1 ± 18.1	18.6 ± 18.7	28.4 ± 16.1
PND 60±2	99.7 ± 51.9	118.6 ± 35.8	107.9 ± 49.7	91.5 ± 43.8
Females				
PND 13	13.3 ± 6.3	16.3 ± 6.0	14.3 ± 6.6	25.2 ± 8.9
PND 17	23.3 ± 14.0	17.7 ± 13.4	22.1 ± 13.7	22.9 ± 7.1
PND 21	21.4 ± 21.9	23.9 ± 20.5	17.0 ± 12.3	16.2 ± 13.4
PND 60±2	97.0 ± 37.8	84.9 ± 37.8	78.9 ± 42.1	97.4 ± 32.9

Data calculated from Table 22, pp. 148-155, MRID 46195601.
[a]Calculated by reviewer from mean interval data.
Session significantly different from control: *$p \leq 0.05$.
N = 10/sex/dose.

TABLE 10. Motor Activity Sub-sessions - male (mean ±S.D. activity counts) [a]

Sub-session (5 min intervals)		Dose (mg/kg/day)			
		0 ppm	0.3 ppm	30 ppm	300 ppm
PND 13	1	9.3±15.9	18.4±18.2	34.4±27	5.3±12.6
	2	9.8±18.2	24.7±29.7	18.7±17.1	6.7±11
	3	11.3±24.1	11.4±17.6	34.5±43.7	3.1±3
	4	6.9±18.7	14.9±21.7	20.6±26.1	8.3±14.1
	5	6.3±12.4	8.7±10.8	15.3±34.1	10.5±15.3
	6	2.6±7.2	9.1±17.9	29.6±31.2	15.7±25.7
	7	1.5±2.2	11.8±20.6	12.3±16.9	9.7±23.5
	8	2.3±3.2	5.9±6.8	16.6±24	4.3±6
	9	2±3.7	4.1±6.6	10.8±16.7	5.5±9.7
	10	1.2±2.8	18.2±30.5	10±12.7	3.9±5
	11	3.1±5.6	15.8±22.8	13.5±20.2	10.3±11.2
	12	11.1±14.3	26.7±44	27.1±21	8.4±16
PND 17∝	1	29.7±30.1	30±35.7	55.5±36.9	56.5±39
	2	28.9±24.5	23.4±31.7	45.8±36.3	44.9±42.1
	3	25.8±33.4	14±11.6	40.2±42.5	52.8±34.4
	4	10±20.2	15.9±20	31.7±38.9	60.8±46.6
	5	18.6±21.5	14.9±23.7	35.9±38.5	40.1±30.6
	6	5.7±7	30.2±44.4	31.4±36.4	46.9±74.9
	7	3.1±5	24.1±35	10.3±24.8	47.9±66.9
	8	13.2±16.1	19.2±30	10.9±25.2	33.6±44
	9	4.1±7	28.4±35.8	9.4±10.2	48.3±80.3
	10	8.4±12.9	9.9±21.2	3.6±3.2	30.8±63.9
	11	6.2±10.3	17.1±31.6	7.2±14	22.5±26.9
	12	5.5±9.8	11.2±26.3	5±10.9	28±33.8
PND 21	1	58.9±35.3	70.2±33.9	65.9±48.9	71±37.3
	2	42.1±41.8	41.6±35.1	41.1±33.7	40.7±21.8
	3	19.5±23.1	21.4±20.6	26.9±27.8	29.7±23.4
	4	14.6±31.1	19.3±28.9	22.2±30.3	22.4±17.6
	5	15.5±20	13.5±21.7	17.3±28.8	6.4±5.9
	6	26.2±31.5	7.3±11	10.5±18.9	28.2±24.8
	7	11.5±21.7	10.1±15.5	9±26.1	36.2±32.8
	8	6.9±10.6	21.1±32	6.5±11.4	26±34.7
	9	5.6±8.7	14.1±23.2	4.3±5.8	17.5±21.4
	10	16.3±27.9	12.3±24.6	0.4±0.7	18.5±22.4
	11	18.2±30.2	16.5±37	5.7±5.8	19.2±23
	12	19.4±31.4	5.3±9.7	13.5±22.3	25.1±24.5
PND 60	1	192.8±46.5	190.4±29.3	219.8±46.3	191.6±35.9
	2	168.3±26.9	161.1±35.3	164.8±27.7	153.4±34.2
	3	146±43.4	156.1±26.1	135.8±35.7	120.1±32.3
	4	129±22	128.5±33	136.7±31.6	100.2±40
	5	110.8±36.2	114.7±25.9	112.6±47.3	85.9±38
	6	101.2±50	108.3±50.1	106.6±46	63.9±34.8
	7	84.1±46.6	92.7±54.5	97.3±43.2	77.1±32.3
	8	79.5±39.9	94.1±37.8	70.8±43.8	68.1±37.3
	9	65±32	12.2±59.9*	55.9±46.2	53.6±44.2
	10	57.3±47.8	106.9±71.4	64.9±47.5	79.7±48.9

TABLE 10. Motor Activity Sub-sessions - male (mean ±S.D. activity counts) [a]

Sub-session (5 min intervals)		Dose (mg/kg/day)			
		0 ppm	0.3 ppm	30 ppm	300 ppm
	11	32.5±31.7	67.8±46.5	60.8±61.7	60.8±45.7
	12	29.4±34.7	80.1±62.4	68.8±56.3	43.5±28.7

a Data obtained from pages 148-154 in the study report MRID 46195601.
N = 10/sex/group
* Statistically different from control at this interval
∝ Significant level difference for the 300 ppm group as compared to control

c. Auditory startle reflex:

AUDITORY STARTLE HABITUATION: Peak amplitude data are summarized in Table 11 and latency data are summarized in Table 12. No treatment-related differences were observed on either testing day.

PRE-PULSE INHIBITION OF STARTLE: Auditory startle pre-pulse inhibition data are given in Table 13. No treatment-related effects were noted in either sex on either testing day. All treated and control groups showed an inhibition of the peak amplitude of response when presented with a pre-pulse before the startle stimulus.

TABLE 11. Auditory startle reflex peak amplitude data (mean volts ±S.D.)

Test day	Block number	0 ppm	0.3 ppm	30 ppm	300 ppm
Males					
PND 23±1	1	22.3 ± 9.5	28.9 ± 6.1	22.9 ± 9.8	20.7 ± 7.5
	2	17.1 ± 7.3	22.4 ± 5.6	21.3 ± 10.0	18.9 ± 5.6
	3	18.7 ± 7.2	21.3 ± 7.8	20.7 ± 9.3	17.6 ± 5.5
	4	18.2 ± 8.2	21.6 ± 6.5	19.2 ± 8.5	17.4 ± 3.7
	5	19.7 ± 10.2	22.1 ± 5.4	16.4 ± 7.8	18.0 ± 4.3
	Overall	19.2 ± 8.1	23.3 ± 5.3	20.1 ± 8.6	18.5 ± 4.3
PND 60±2	1	72.5 ± 35.5	73.6 ± 29.6	49.4 ± 19.5	75.3 ± 46.9
	2	43.2 ± 15.7	55.8 ± 24.8	31.0 ± 12.6	41.5 ± 25.7
	3	42.9 ± 14.7	42.2 ± 16.1	27.7 ± 12.9	37.3 ± 19.7
	4	36.9 ± 12.6	44.6 ± 22.6	31.1 ± 18.3	30.4 ± 13.7
	5	37.8 ± 19.8	43.9 ± 15.7	26.7 ± 16.5	34.2 ± 12.4
	Overall	46.7 ± 16.0	52.0 ± 17.4	33.2 ± 13.8	43.7 ± 20.8
Females					
PND 23±1	1	25.5 ± 12.4	21.7 ± 7.5	22.3 ± 12.4	25.1 ± 8.5
	2	21.9 ± 11.4	19.5 ± 8.7	19.7 ± 14.7	21.8 ± 6.3
	3	21.5 ± 8.5	18.2 ± 5.9	18.5 ± 12.2	18.8 ± 4.4
	4	20.2 ± 13.0	18.5 ± 6.2	17.1 ± 11.2	19.3 ± 3.5
	5	19.3 ± 10.4	21.0 ± 7.0	16.5 ± 9.3	18.7 ± 8.2
	Overall	21.7 ± 10.7	19.8 ± 6.4	18.8 ± 11.6	20.7 ± 5.0
PND 60±2	1	57.1 ± 26.7	40.1 ± 22.9	37.6 ± 20.9	53.7 ± 24.3
	2	45.7 ± 22.8	27.7 ± 13.4	26.4 ± 15.6	39.0 ± 21.8
	3	44.8 ± 19.4	28.7 ± 15.3	23.4 ± 18.4	37.4 ± 18.2
	4	46.1 ± 25.2	28.4 ± 15.8	27.5 ± 16.5	39.2 ± 31.6
	5	43.9 ± 31.2	23.6 ± 10.1	30.3 ± 20.8	32.3 ± 19.0
	Overall	47.5 ± 23.7	29.7 ± 14.7	29.0 ± 17.3	40.3 ± 20.5

Data obtained from Table 23, pp. 160-163, MRID 46195601.
N = 10/sex/dose
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 12. Auditory startle reflex latency data (mean msec ±S.D.)

Test day	Block number	0 ppm	0.3 ppm	30 ppm	300 ppm
Males					
PND 23±1	1	30.3 ± 4.5	31.5 ± 5.7	25.1 ± 3.4	28.9 ± 6.0
	2	28.4 ± 3.9	29.0 ± 3.8	26.4 ± 4.8	28.5 ± 4.0
	3	29.5 ± 6.7	27.7 ± 3.2	24.8 ± 3.6	28.6 ± 2.9
	4	28.4 ± 5.2	25.7 ± 3.4	24.2 ± 2.5	29.5 ± 3.3
	5	28.7 ± 6.1	26.6 ± 3.1	25.5 ± 3.4	28.3 ± 3.9
	Overall	29.1 ± 3.7	28.1 ± 2.5	25.2* ± 2.3	28.8 ± 2.9
PND 60±2	1	29.8 ± 7.4	28.0 ± 3.9	27.8 ± 3.1	27.2 ± 5.5
	2	26.0 ± 3.0	25.7 ± 3.6	30.2 ± 9.5	24.4 ± 2.4
	3	26.8 ± 4.5	27.7 ± 5.3	27.5 ± 6.7	27.9 ± 4.8
	4	27.0 ± 5.4	27.0 ± 5.1	29.6 ± 7.4	26.0 ± 4.2
	5	25.2 ± 2.9	28.1 ± 8.8	32.5 ± 10.8	27.3 ± 8.9
	Overall	26.9 ± 2.6	27.3 ± 3.6	29.5* ± 6.4	26.6 ± 3.2
Females					
PND 23±1	1	25.4 ± 5.4	28.1 ± 3.8	33.4 ± 13.2	29.2 ± 4.1
	2	25.8 ± 4.0	26.3 ± 2.2	33.5 ± 11.0	27.7 ± 5.0
	3	27.3 ± 5.0	26.0 ± 2.3	33.1 ± 11.3	27.0 ± 4.6
	4	26.5 ± 4.0	24.7 ± 4.0	30.3 ± 6.4	26.1 ± 5.5
	5	25.2 ± 5.4	25.6 ± 4.9	30.7 ± 7.3	28.1 ± 6.4
	Overall	26.0 ± 3.6	26.1 ± 2.5	32.2 ± 8.4	27.6 ± 4.3
PND 60±2	1	27.8 ± 3.9	26.8 ± 4.6	32.0 ± 9.7	25.6 ± 4.8
	2	26.5 ± 4.7	27.1 ± 3.5	28.4 ± 5.7	23.7 ± 2.6
	3	24.2 ± 3.0	26.0 ± 4.5	29.6 ± 8.5	23.6 ± 4.4
	4	28.2 ± 9.2	28.2 ± 8.0	33.4 ± 10.2	26.0 ± 4.8
	5	27.6 ± 5.4	27.0 ± 5.0	26.7 ± 3.8	27.6 ± 6.0
	Overall	26.8 ± 3.9	27.0 ± 3.0	30.0 ± 6.3	25.3 ± 3.4

Data obtained from Table 23. pp. 156-159. MRID 46195601.
*Noted as statistically significant in the text but not on the data table.
N = 10/sex/dose

TABLE 13. Auditory startle reflex pre-pulse inhibition data (mean ±S.D.)[a]				
Pre-pulse	**0 ppm**	**0.3 ppm**	**30 ppm**	**300 ppm**
Males PND 23				
No	22.6 ± 8.56	26.8 ± 9.64	24.6 ± 7.37	18.5 ± 2.87
Yes	15.3 ± 7.22	20.1 ± 8.52	15.2 ± 5.73	13.2 ± 3.50
% Inhibition	33.7 ± 13.87	26.4 ± 11.23	36.2 ± 20.32	28.7 ± 14.66
Females PND 23				
No	29.3 ± 9.79	20.0 ± 8.73	23.3 ± 5.92	22.7 ± 8.56
Yes	22.4 ± 6.71	14.3 ± 7.86	16.6 ± 3.70	16.5 ± 5.73
% Inhibition	21.8 ± 18.21	30.5 ± 25.74	26.9 ± 13.90	24.9 ± 14.29
Males PND 60±2				
No	56.5 ± 23.87	81.0 ± 31.86	50.2 ± 18.45	64.0 ± 33.04
Yes	18.9 ± 10.62	45.0 ± 31.75	21.6 ± 13.90	27.9 ± 15.75
% Inhibition	65.8 ± 16.95	49.7 ± 20.14	57.7 ± 13.99	54.5 ± 22.81
Females PND 60±2				
No	51.3 ± 15.73	40.2 ± 23.67	33.6 ± 8.32	41.1 ± 14.60
Yes	28.5 ± 16.02	17.6 ± 9.98	14.8 ± 4.57	18.5 ± 12.19
% Inhibition	45.6 ± 19.69	53.7 ± 13.90	53.7 ± 18.66	55.7 ± 18.94

Data obtained from Table 23, pp. 165-168, MRID 46195601.
[a]Units of measurement were not given with these data; unit is assumed to be volts as a measure of the amplitude of response since this is what was used in auditory startle habituation.
N = 10/sex/dose

d. **Learning and memory testing:** Performance results in the Biel maze are given in Tables 14 and 15 for males and females, respectively. Males from the high-dose group had significantly longer swimming time and a greater number of errors on the first and second days of testing on both PND 24 and 60. This appears to be treatment-related. In females, there was an increase in latency and errors on test days 1 and 2 at the mid dose on PND 24 only. Due to lack of dose response, the results in females were not considered to be treatement-related. No other treatment-related effects on learning and memory were observed in males or females. All groups demonstrated learning of the position of the escape platform as evidenced by decreases in trial time and the number of errors over the four days of testing.

TABLE 14. Biel swimming trials - males (mean ± S.D.)					
Test Day	**Parameter**	**0 ppm**	**0.3 ppm**	**30 ppm**	**300 ppm**
PND 24					
Initial	Swimming ability (sec)	8.5 ± 2.70	9.5 ± 2.55	9.8 ± 3.70	7.7 ± 2.61
Test day 1	Time (sec)	62.1 ± 37.3	57.6 ± 36.65	69.8 ± 29.65	86.8 ± 52.28
	Errors	12.4 ± 7.59	10.8 ± 6.72	14.3 ± 7.01	15.3 ± 8.54
Test day 2	Time (sec)	33.9 ± 18.06	39.5 ± 36.14	29.4 ± 13.51	**62.1* ± 36.40**
	Errors	7.2 ± 5.55	8.7 ± 10.51	5.6 ± 3.36	**12.6 ± 8.37**
Test day 3	Time (sec)	30.7 ± 15.65	30.0 ± 11.79	29.1 ± 18.81	31.8 ± 24.6
	Errors	7.1 ± 4.99	5.8 ± 3.16	5.5 ± 5.30	6.0 ± 6.36
Test day 4	Time (sec)	27.9 ± 19.89	32.7 ± 15.96	29.6 ± 18.01	27.3 ± 19.87
	Errors	6.5 ± 6.77	7.3 ± 6.26	6.5 ± 5.78	5.6 ± 6.29
Recall	Time (sec)	34.3 ± 26.73	30.2 ± 11.16	21.1 ± 12.67	22.5 ± 8.30
	Errors	7.4 ± 8.07	5.9 ± 3.16	3.2 ± 3.99	3.8 ± 2.81
PND 60					
Initial	Swimming ability (sec)	10.7 ± 8.69	8.0 ± 1.96	9.2 ± 1.92	7.7 ± 3.64
Test day 1	Time (sec)	61.3 ± 37.91	57.6 ± 30.86	44.8 ± 20.32	47.7 ± 20.25
	Errors	12.9 ± 6.62	12.0 ± 5.79	9.4 ± 5.81	9.3 ± 5.17
Test day 2	Time (sec)	26.9 ± 14.36	33.0 ± 10.07	39.2 ± 20.40	**57.3* ± 46.84**
	Errors	5.8 ± 4.35	8.2 ± 3.41	7.6 ± 5.20	**13.2 ± 12.81**
Test day 3	Time (sec)	32.3 ± 16.26	25.8 ± 6.53	22.5 ± 13.09	29.6 ± 19.2
	Errors	7.9 ± 4.73	5.8 ± 2.53	3.9 ± 3.65	6.1 ± 5.85
Test day 4	Time (sec)	22.5 ± 6.30	21.6 ± 7.50	23.2 ± 16.25	32.6 ± 17.86
	Errors	4.7 ± 2.30	4.1 ± 1.79	3.9 ± 2.48	7.5 ± 5.37
Recall	Time (sec)	19.3 ± 7.31	17.8 ± 5.97	19.2 ± 8.41	22.2 ± 9.65
	Errors	3.3 ± 2.55	3.2 ± 2.93	2.3 ± 1.81	4.0 ± 2.76

Data obtained from Table 21, pp. 144-147, MRID 46195601.
Significantly different from control: *p ≤ 0.05
N = 10/group

TABLE 15. Biel swimming trials - females (mean ± S.D.)					
Test Day	**Parameter**	**0 ppm**	**0.3 ppm**	**30 ppm**	**300 ppm**
PND 24					
Initial	Swimming ability (sec)	8.4 ± 2.80	9.0 ± 3.64	9.3 ± 2.72	9.4 ± 3.11
Test day 1	Time (sec)	46.9 ± 32.29	54.5 ± 31.12	73.1 ± 38.93	63.0 ± 46.78
	Errors	8.9 ± 7.62	11.8 ± 6.50	15.4 ± 8.68	11.7 ± 7.92
Test day 2	Time (sec)	35.7 ± 16.66	37.6 ± 7.78	69.0** ± 23.25	41.2 ± 13.49
	Errors	7.7 ± 5.38	9.3 ± 3.08	17.4** ± 6.75	9.4 ± 3.79
Test day 3	Time (sec)	35.0 ± 22.63	44.5 ± 33.23	24.4 ± 13.85	40.8 ± 36.93
	Errors	8.2 ± 6.48	11.3 ± 9.41	5.2 ± 4.50	8.0 ± 8.50
Test day 4	Time (sec)	21.4 ± 8.16	23.5 ± 12.88	33.4 ± 23.33	28.5 ± 24.19
	Errors	4.9 ± 3.18	4.5 ± 3.81	7.7 ± 6.47	6.7 ± 9.04
Recall	Time (sec)	23.0 ± 9.83	32.6 ± 21.25	25.8 ± 13.33	25.0 ± 11.46
	Errors	4.4 ± 2.33	6.4 ± 5.97	4.7 ± 4.18	4.8 ± 3.82
PND 60					
Initial	Swimming ability (sec)	10.7 ± 4.52	9.7 ± 2.69	10.1 ± 6.69	10.3 ± 3.42
Test day 1	Time (sec)	65.6 ± 43.17	96.1 ± 22.97	76.5 ± 37.39	56.3 ± 23.71
	Errors	16.1 ± 12.14	20.9 ± 5.58	15.7 ± 6.26	11.6 ± 5.94
Test day 2	Time (sec)	34.8 ± 18.27	47.4 ± 32.02	30.4 ± 17.46	35.2 ± 18.71
	Errors	8.7 ± 6.40	9.7 ± 6.15	5.9 ± 3.57	7.2 ± 4.22
Test day 3	Time (sec)	26.9 ± 11.41	27.9 ± 13.60	28.8 ± 15.29	33.9 ± 23.95
	Errors	6.4 ± 4.32	5.1 ± 3.25	6.1 ± 4.03	6.4 ± 6.10
Test day 4	Time (sec)	21.6 ± 11.16	32.4 ± 18.89	26.6 ± 14.19	33.5 ± 29.58
	Errors	4.2 ± 3.77	7.1 ± 5.73	5.1 ± 4.30	6.9 ± 8.16
Recall	Time (sec)	21.7 ± 9.80	28.0 ± 16.76	30.2 ± 18.72	25.6 ± 14.67
	Errors	4.1 ± 3.20	4.9 ± 3.69	5.0 ± 3.27	3.9 ± 3.22

Data obtained from Table 21, pp. 144-147, MRID 46195601.
Significantly different from control: **p ≤ 0.01
N = 10/group

e. **Ophthalmology:** Complete ophthalmoscopic examinations were not done.

5. **Postmortem results:**

a. **Brain weights:** Mean brain weight data are presented in Table 16. No treatment-related effect on absolute brain weight was observed at weaning or study termination.

TABLE 16. Mean (±SD) brain weight data in offspring				
Parameter	**0 ppm**	**0.30 ppm**	**30 ppm**	**300 ppm**
Males				
PND 21				
Terminal body weight (g)	49.7 ± 5.21	49.3 ± 5.78	50.5 ± 3.68	42.2 ± 8.88
Brain-to-body weight ratio	0.029 ± 0.002	0.030 ± 0.003	0.029 ± 0.002	0.034** ± 0.006
PND 60				
Terminal body weight (g)	381.6 ± 21.37	366.1 ± 31.43	391.8 ± 32.85	338.0 ± 32.53
Brain weight (g)	2.05 ± 0.10	2.04 ± 0.12	2.06 ± 0.19	1.93 ± 0.14
Females				
PND 21				
Terminal body weight (g)	47.5 ± 4.87	46.2 ± 6.31	48.4 ± 4.43	36.9 ± 4.73
Brain weight (g)	1.36 ± 0.05	1.40 ± 0.06	1.40 ± 0.06	1.34 ± 0.05
PND 60				
Terminal body weight (g)	224.2 ± 15.99	227.9 ± 13.59	228.6 ± 15.36	229.3 ± 20.78
Brain weight (g)	1.86 ± 0.13	1.96 ± 0.13	1.85 ± 0.10	1.91 ± 0.08

Data obtained from Table 28, pp. 188-191, MRID 46195601.
N = 10/sex/dose
Significantly different from control: **p ≤ 0.01.

b. **Cholinesterase activity:** Cholinesterase activity for dams and pups is given in Table 17. In mid- and high-dose dams, cholinesterase activity was significantly inhibited in all three compartments: enzyme activity was inhibited by 67 and 92.6%, respectively, in plasma, by 87.3 and 100%, respectively, in RBC, and by 24.8 and 86.8%, respectively, in brain. No inhibition was measured in low-dose dams.

In 4-day old male and female pups from high-dose litters, cholinesterase activity was significantly inhibited in plasma by 50.1 and 48.4%, respectively, in RBC by 41.3 and 37.8%, respectively, and in brain by 16.7 and 13.4%, respectively. No effects on cholinesterase activity were found in pups from the low- and mid-dose groups.

On PND 21 dose-related inhibition of cholinesterase activity was observed in male and female pups from the mid- and high-dose litters. Plasma enzyme activity was inhibited in the mid- and high-dose offspring by 34.4 and 68.2%, respectively, in males and by 18.6 and 51.2%, respectively, in females. RBC enzyme activity was inhibited in mid- and high-dose males by 23.5 (not statistical) and 58.3%, respectively, and in high-dose females by 53.8%. Males and females from the high-dose group had 44.2 and 28.6% inhibition, respectively, of brain enzyme activity.

TABLE 17. Cholinesterase activity in dams and offspring [mean ± SD (% inhibition relative to control)]

Compartment	0 ppm	0.30 ppm	30 ppm	300 ppm
PND 21 dams				
Plasma (IU/mL)	0.76 ± 0.10	0.73 ± 0.15 (3.3)	0.25** ± 0.05 (67.0)	0.06** ± 0.02 (92.6)
RBC (IU/mL)	1.34 ± 0.20	1.49 ± 0.29	0.18** ± 0.28 (87.3)	0.00 ± 0.00 (100)
Brain (IU/mL) [1]	15.30 ± 0.52	15.69 ± 0.82	11.50** ± 1.63 (24.8)	2.02* ± 0.23 (86.8)
PND 4 males				
Plasma (IU/mL)	0.97 ± 0.31	0.82 ± 0.10 (15.2)	0.86 ± 0.17 (11)	0.48** ± 0.05 (50.1)
RBC (IU/mL)	2.74 ± 0.45	2.37 ± 0.43 (13.4)	2.42 ± 0.43 (11.4)	1.61** ± 0.49 (41.3)
Brain (IU/mL)	4.64 ± 0.59	4.47 ± 0.50 (3.8)	4.57 ± 0.63 (1.6)	3.87** ± 0.34 (16.7)
PND 4 females				
Plasma (IU/mL)	0.89 ± 0.09	0.80 ± 0.13 (10.2)	0.82 ± 0.19 (7.4)	0.46** ± 0.11 (48.4)
RBC (IU/mL)	2.41 ± 0.36	2.45 ± 0.64	2.45 ± 0.36	1.50** ± 0.54 (37.8)
Brain (IU/mL)	4.78 ± 0.53	5.03 ± 0.50	4.79 ± 0.60	4.14** ± 0.36 (13.4)
PND 21 males				
Plasma (IU/mL)	0.76 ± 0.11	0.68 ± 0.12 (10.6)	0.50** ± 0.05 (34.4)	0.24** ± 0.08 (68.2)
RBC (IU/mL)	3.85 ± 1.02	3.61 ± 0.79 (6.2)	2.95 ± 0.81 (23.5)	1.60** ± 1.22 (58.3)
Brain (IU/mL)	13.93 ± 0.85	13.81 ± 0.99 (0.8)	13.87 ± 1.14 (0.4)	7.77** ± 2.53 (44.2)
PND 21 females				
Plasma (IU/mL)	0.63 ± 0.10	0.69 ± 0.09	0.51* ± 0.10 (18.6)	0.31** ± 0.17 (51.2)
RBC (IU/mL)	3.52 ± 1.11	3.08 ± 1.02 (12.5)	3.23 ± 0.66 (8.2)	1.63** ± 1.06 (53.8)
Brain (IU/mL)	13.86 ± 1.22	13.84 ± 0.91 (0.2)	13.33 ± 2.11 (3.9)	9.90** ± 3.35 (28.6)

Data obtained from Table 24, pp. 170-174, MRID 46195601.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.
[1] Brain volume of homogenate was used. There was no indication that values were normalized based on weight of the sample.

c. **Neuropathology**:

1) **Macroscopic examination:** No treatment-related effects were reported for male or female offspring at PNDs 21 or 60.

2) **Microscopic examination:** No significant treatment-related effects were noted on PNDs 21 or 60. At study termination, retinal foldings in the eye were observed in one control female, in one high-dose female, and in two high-dose males. No treatment-related effects were observed on the tissues of the central or peripheral nervous system; minimal fiber degeneration was seen in the sciatic nerve from one control male and the tibial nerve from one high-dose male.

3) **Brain Morphometry:** Data for males and females are summarized in Table 18. No treatment-related differences in any measurement were observed for either sex at PNDs 21 or 65.

TABLE 18. Mean (±SD) morphometric data in offspring				
Parameter	**0 ppm**	**0.30 ppm**	**30 ppm**	**300 ppm**
Males PND 21				
Neocortex (mm)	1.5 ± 0.13	--	--	1.5 ± 0.10
Hippocampus (mm)	1.3 ± 0.06	--	--	1.3 ± 0.16
Cerebellum (mm)	0.7 ± 0.05	--	--	0.7 ± 0.03
Females PND 21				
Neocortex (mm)	1.4 ± 0.13	--	--	1.4 ± 0.14
Hippocampus (mm)	1.3 ± 0.08	--	--	1.3 ± 0.08
Cerebellum (mm)	0.7 ± 0.07	--	--	0.7 ± 0.06
Males PND 60				
Neocortex (mm)	1.4 ± 0.12	--	--	1.4 ± 0.11
Hippocampus (mm)	1.6 ± 0.08	--	--	1.6 ± 0.07
Cerebellum (mm)	0.8 ± 0.05	--	--	0.7 ± 0.04
Females PND 60				
Neocortex (mm)	1.5 ± 0.12	--	--	1.4 ± 0.10
Hippocampus (mm)	1.6 ± 0.11	--	--	1.6 ± 0.11
Cerebellum (mm)	0.7 ± 0.06	--	--	0.7 ± 0.09

Data obtained from Table 27, pp. 186-187, MRID 46195601.
N = 10/sex/dose

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that maternal toxicity was evident at 30 and 300 ppm as inhibition of plasma, RBC, and brain cholinesterase activities. Additional findings in dams at 300 ppm included minimal clinical observations and changes in food consumption. In pups, cholinesterase activity was inhibited in all three compartments on PNDs 4 and 21 at 300 ppm and in plasma on PND 21 at 30 ppm. Additionally, in pups at 300 ppm findings included decreases in body weight and body weight gain, and delays in appearance of developmental landmarks. The NOAELs for dams and pups were specified as 0.3 ppm and 30 ppm, respectively.

B. **REVIEWER COMMENTS:** In dams, no treatment-related effects on mortality, clinical signs, body weight, FOB parameters, or necropsy findings were noted. The reviewer does not think that the observation of piloerection in a few high-dose animals on one day is clear evidence of maternal toxicity. There was a slight decrease in body weight gain and food consumption in the high dose dams. This is supported by the occurrence of cholinesterase inhibition in all three compartments at the mid dose, and to a greater extent, the high dose (discussed below).

No treatment-related effect on the reproductive parameters or offspring survival was observed. Significant growth retardation occurred in male and female pups from the high-dose group. Although body weight at birth was similar between the treated and control groups, the high-dose pups gained less weight throughout lactation resulting in reduced absolute body weight by PND 7 for males and PND 4 for females. During the post-weaning interval, recovery of body weight was apparent for females but not for males. Corresponding

with the growth retardation, sexual maturation was delayed in male and female offspring from the high-dose litters compared with the controls.

In the assessment of motor activity, there was a possible treatment related effect in the high dose males on PND 17, however, it was difficult to interpret the biological significance of this since it only occurred on one day and there was large variability. It was also noted that activity for PND 21 was highly variable across intervals for both males and females. Overall, it was determined that the motor activity assessment was inadequate and difficult to interpret because of too much variability in the data.

In the assessment of auditory startle response, males from the high-dose group had a longer swimming time and a greater number of errors on the first and second day of testing on both PND 24 and 60 compared with the controls. Due to a lack of dose response, the increase in latency and errors seen on test days 1 and 2 in females at the mid dose on PND 24 was not considered to be treatment-related.

No treatment-related effect on absolute brain weight, microscopic examination, or morphometry was observed at weaning or study termination..

Plasma, RBC, and brain cholinesterase activities were statistically and biologically inhibited in mid- and high-dose dams on PND 21 and in the high-dose pups on PND 4. On PND 21 inhibition of enzyme activity was evident in all three compartments from high-dose offspring, in plasma from mid-dose males and females and in RBC from mid-dose males. Although inhibition of RBC enzyme activity for the mid-dose males on PND 21 was not statistically significant and only marginally biologically significant, a clear dose-response was evident. Inhibition was more pronounced in dams than in offspring on day 21.

For maternal systemic toxicity, the NOAEL is 30 ppm (2.36 mg/kg/day), the highest dose tested. A LOAEL was not established.

For maternal cholinesterase inhibition, the NOAEL is 0.3 ppm (0.026 mg/kg/day). The LOAEL is 30 ppm (2.36 mg/kg/day) based on decreases in plasma, RBC and brain cholinesterase activities.

For offspring systemic toxicity, the NOAEL is 30 ppm (2.36 mg/kg/day). The LOAEL is 300 ppm (24.2 mg/kg/day) based reduced body weight and body weight gain an delayed sexual maturation in males and females.

For offspring cholinesterase NOAEL is 0.30 ppm (0.026 mg/kg/day). The LOAEL is 30 ppm (2.36 mg/kg/day) based on decreases in plasma and RBC cholinesterase activities in both sexes.

This study is classified **Acceptable/NonGuideline** 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) due to the inadequacies in the assessment of motor activity in the offspring and the pending review of the of positive control data.

C. **STUDY DEFICIENCIES:** Dams with whole litter loss should have been included in calculations of some reproductive parameters. There was no information regarding the conditions of the open arena observations.

APPENDIX

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

PC CODE: 057801 **DP BARCODE:** D299890

TEST MATERIAL (PURITY): Diazinon Technical (94.2%)

SYNONYMS: 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether

CITATION: Mandella, R.C. (2002) Diazinon: a dietary range-finding developmental neurotoxicity study in rats. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study no. 01-4530; November 13, 2002. MRID 45842601. Unpublished.

SPONSOR: Makhteshim-Agan of North America, 551 Fifth Avenue, Suite 1100, New York, NY 10176

EXECUTIVE SUMMARY: In a range-finding developmental neurotoxicity study (MRID 45842601), Diazinon technical (94.2% a.i., batch # 9896144) was administered to 10 female Sprague-Dawley CD® rats/dose in the diet at concentrations of 0, 0.1, 0.5, 50, or 300 ppm from gestation day (GD) 6 through postnatal day (PND) 21. The average daily test article intake during the study was 0, 0.0125, 0.063, 6.56, and 38.06 mg/kg/day. A Functional Operational Battery (FOB) was performed on 5 dams/dose on GDs 7, 14, and 20, and on PNDs 1, 4, 7, 14, and 20. On PND day 4, litters were culled to yield five males and five females (as closely as possible). The FOB was conducted on 8 pups/sex/group on PND 4, 7, 11, 14, 17, and 20. Blood and brain cholinesterase activities were measured in 8 pups/sex/group on PND 4 and 21, in 2 dams/group on PND 5 or 6, and in 8 dams/group on PND 21.

All dams survived to scheduled sacrifice. During gestation, no effects on body weight or body weight gain were noted. During lactation, body weight gain by the 300-ppm dams was decreased by 25-80% of the control level. Food consumption was similar between the treated groups and the control group during gestation and lactation. At 300 ppm treatment-related observations during the maternal FOB included tremors in two animals and absence of pupil response in two animals. Maternal necropsy was unremarkable.

The maternal systemic LOAEL is 300 ppm (38.06 mg/kg/day) based on clinical signs of toxicity and the NOAEL is 50 ppm (6.56 mg/kg/day).

Treatment did not affect duration of gestation, number of liveborn pups, number of pups/litter, or pup survival. At 300 ppm female pups had an equivocal lag in the development of the surface righting reflex. Body weight and body weight gain for male and female pups in the 300 ppm group were decreased throughout lactation. Absolute body weight of the 300-ppm pups was significantly less than that of controls beginning on PND 11. The most pronounced effect on weight gain in the 300-ppm pups was during PND 4-11 when gains were decreased by 39-42% of the control level. No treatment-related abnormalities were found at gross necropsy of the pups.

The offspring systemic LOAEL is 300 ppm (38.06 mg/kg/day) based on decreased body weight and body weight gain and the NOAEL is 50 ppm (6.56 mg/kg/day).

At dietary concentrations ≥0.5 ppm, dose-related inhibition of blood and brain cholinesterase activities was apparent in dams on PND 21. For the 0.5, 50, and 300 ppm groups plasma enzyme activity was inhibited by 45.8, 79.7, and 93.9%, respectively, red cell enzyme activity was inhibited by 39.3, 95.9, and 100%, respectively, and brain enzyme activity was inhibited by 8.6, 43.0, and 89.2%, respectively, compared with the control levels.

For male and female pups from the 300 ppm group, plasma cholinesterase activity was inhibited by 33.4 and 29.6%, respectively, on PND 4 and by 48.2 and 45.8%, respectively, on PND 21. Male and female pups in the 50 ppm group had plasma cholinesterase activity inhibited by 34.6 and 40.1%, respectively. For male and female pups from the 300 ppm group, red cell cholinesterase activity was inhibited by 44.5 and 48.9%, respectively, on PND 4 and by 48.2 and 57.4%, respectively, on PND 21; and brain cholinesterase activity was inhibited by 16.8 and 18.1%, respectively, on PND 4 and by 36.7 and 37.2%, respectively, on PND 21.

The LOAEL for plasma cholinesterase inhibition in dams is 0.5 ppm (0.063 mg/kg/day) and the NOAEL is 0.1 ppm (0.0125 mg/kg/day). The LOAEL for plasma cholinesterase inhibition in offspring is 50 ppm (6.56 mg/kg/day) and the NOAEL is 0.5 ppm (0.063 mg/kg/day).

The LOAEL for red cell cholinesterase inhibition in dams is 50 ppm (6.56 mg/kg/day) and the NOAEL is 0.5 ppm (0.063 mg/kg/day). The LOAEL for red cell cholinesterase inhibition in offspring is 300 ppm (38.06 mg/kg/day) and the NOAEL is 50 ppm (6.56 mg/kg/day).

The LOAEL for brain cholinesterase inhibition in dams is 50 ppm (6.56 mg/kg/day) and the NOAEL is 0.5 ppm (0.063 mg/kg/day). The LOAEL for brain cholinesterase inhibition in offspring is 300 ppm (38.06 mg/kg/day) and the NOAEL is 50 ppm (6.56 mg/kg/day).

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