DATA EVALUATION RECORD

UNDATED

(YRC 2894) THIACLOPRID

Study Type: §83-6, Developmental Neurotoxicity Study in Rats

Work Assignment No. 4-02-179 (formerly 4-01-179) MRID 45516601

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

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

PC CODE: 014019 **DP BARCODE:** D281298
SUBMISSION NO.: S604757

TEST MATERIAL (PURITY): YRC 2894 (Thiacloprid; 99.2% a.i.)

SYNONYMS: (3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]cyanamide

CITATION: Hoberman, A.M. (2001) Oral (Diet) Developmental Neurotoxicity Study of YRC 2894 in CRL:CD®(SD)IGS BR VAF/PLUS®. Argus Research Laboratories, Inc., Horsham, PA. Laboratory Project ID.: 99C-D72-ER, September 24, 2001. MRID 45516601. Unpublished.

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

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45516601) YRC 2894 (Thiacloprid; 99.2% a.i., 898013001) was administered to 25 female Crl:CD®(SD)IGS BR VAF/Plus® rats per dose in the diet at dose levels of 0, 50, 300, or 500 ppm (0, 4.4, 25.6, and 40.8 mg/kg/day during gestation; 0, 8.2, 49.4, and 82.8 mg/kg/day during lactation) from gestation day (GD) 0 through lactation day (LD) 22. The day that litter delivery was completed was designated postnatal day (PND) 1 (or LD 1). Body weight and food consumption data were recorded for dams. Detailed clinical observations, including assessments of autonomic function, were conducted daily during gestation and on LD 1, 5, 8, 14, and 22. Dams were killed and necropsied on LD 22. On PND 5, litters were standardized to yield 5 males and 5 females (as closely as possible), and 10 randomly selected pups/sex/group were subjected to detailed clinical examination outside the home cage. On PND 12, pups were randomly assigned to each of the following four subsets: 1) fixed brain weights and/or neuropathological evaluation on PND 12 (10/sex/group); 2) passive avoidance testing (on PND 23-25 and 30-32) and water maze testing (on PND 59-63 and 66-70) (20/sex/group); 3) motor activity testing (on PND 14, 18, 22, and 58-60) and auditory startle habituation (on PND 23 and 59-61) (20/sex/group); 4) detailed clinical exam outside the home cage on PND 12 and weekly during the postweaning period (20/sex/group), fixed brain weights and neuropathological evaluation on PND 68-79

(10/sex/group). In addition, the pups from subsets 2-4 were observed for the age of attainment of balanopreputial separation or vaginal patency (60/sex/group).

There were no treatment-related effects on maternal survival, clinical or functional observations, reproductive function, or gross pathology at any dietary level. Treatment-related decreases ($p \leq 0.05$) in maternal body weight gain were observed in the 300 and 500 ppm dams during GDs 0-6 (↓31-56%), and in the 500 ppm dams during LDs 1-4 (↓67%). Treatment-related decreases in food consumption ($p \leq 0.01$) were also noted in the 300 and 500 ppm dams during GDs 0-6 (↓16-30%) and in the 500 ppm dams during LD 4-7 (↓11%). Significant decreases ($p \leq 0.01$) in relative food consumption on GD 0-6 at 300 (↓14%) and 500 ppm (↓27%) support the conclusion that the effects on body weight gain in early gestation were not solely related to palatability.

The maternal LOAEL is 300 ppm (25.6 mg/kg/day), based on decreased body weight gain and food consumption during early gestation (GD 0-6). The maternal NOAEL is 50 ppm (4.4 mg/kg/day).

Offspring survival, assessments of autonomic function, watermaze, brain weights, and qualitative histopathology were unaffected by treatment. Suggestive effects on motor activity and auditory startle were seen in the 300 and 500 ppm groups. Increased incidences ($p \leq 0.01$) of malaligned incisors and chromodacryorrhea were observed at postpartum week 10 in 500 ppm offspring. These findings were considered to be treatment-related, perhaps a latent expression of a developmental anomaly. In the 300 and 500 ppm offspring, preweaning body weights were decreased ($p \leq 0.01$) on PNDs 8-22 (↓5-15%). In addition, postweaning body weights were decreased ($p \leq 0.01$) in these animals (↓4-15%). Sexual maturation was delayed ($p \leq 0.05$) in the 300 and 500 ppm male pups (48.2 days each treated vs. 46.7 days controls), and in the 500 ppm female pups (34.7 days treated vs. 33.4 days controls).

Passive avoidance testing revealed significant increases in Trial 2 latency during the first testing session in 300 and 500 ppm females ($p \leq 0.05$ and $p \leq 0.01$, respectively) in weanling offspring. Also at these doses, examination of the individual data indicated slower responses, and an adverse effect on retention of behaviors learned in Session 1.

At histopathological evaluation, in the 500 ppm males, the size of the corpus striatum (↓4%) and corpus callosum (↓14%) were decreased ($p \leq 0.05$) from controls on PND 12. At study termination, the corpus striatum (↓4%) and dentate gyrus (↓5%) were smaller ($p \leq 0.05$) than controls. A definitive NOAEL was not established for these findings.

The offspring LOAEL is tentatively set at 300 ppm (25.6 mg/kg/day), based on decreased preweaning and postweaning body weights in both sexes and delayed sexual maturation in the males, and altered performance in passive avoidance testing. The tentative offspring NOAEL is 50 ppm (4.4 mg/kg/day).

This study is classified **acceptable/nonguideline** and does not satisfy the guideline requirement

for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). This study can be upgraded following the submission of acceptable morphometric histopathology data to establish a definitive NOAEL for alterations in brain development, procedural information for functional observation assessments, and adequate positive control data.

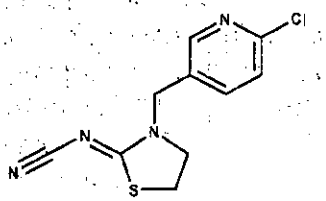
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: YRC 2894

Description:	Yellowish powder
Lot/Batch #:	898013001
Purity:	99.2% a.i.
Compound Stability:	It was stated that the test substance was stable in the diet.
CAS # of TGAI:	111988-49-9



2. Vehicle and/or positive control: Diet; 1% w/w corn oil

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®	
Age at study initiation:	Approximately 10 wks	
Wt. at study initiation:	216-260 g (females)	
Source:	Charles River Laboratories, Inc., Portage, MI	
Housing:	Individually, in stainless steel wire-bottomed cages except during mating and lactation. By litter in nesting boxes with Bed-o'cobs® bedding during late gestation and through lactation.	
Diet:	Certified Rodent Diet #5001-4 (PMI Nutrition International, St. Louis, MO), *ad libitum*	
Water:	Reverse osmosis tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C (nominal and actual)
	Humidity:	30-70% (nominal only; see Study Deficiencies at end of DER)
	Air changes:	≥ 10/hour
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	5 days	

4

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 9/5/2000 End: 12/22/2000

2. Study schedule: The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day (GD) 0 through lactation day (LD) 22. Pups were weaned on postnatal day 22, after which time maternal animals were killed. F1 pups were assigned to subgroups in order to evaluate functional behavioral endpoints, motor activity, auditory learning and memory, startle response brain weights, and neuropathology.

3. Mating procedure: Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. No later than GD 20, dams were individually housed in nesting boxes, where they were maintained through lactation.

4. Animal Assignment: Mated females were randomly assigned, stratified by body weights, to dose groups as indicated in Table 1. Offspring originating from 20 of the approximately 25 available litters/group were randomly assigned to testing subgroups on postnatal day 12 (Table 1). Litters of fewer than 9 pups were not retained.

Table 1. Study design [a]

Experimental Parameter	Subgroup	Dose (ppm)			
		0	50	300	500
Maternal Animals					
No. of maternal animals assigned	NA	25	25	25	25
Detailed clinical observations (daily from GD 0 through LD 22)	NA	25	25	25	25
Offspring					
Detailed clinical observations (PND 12)	2, 3, 4	3pups/sex/litter	3pups/sex/litter	3pups/sex/litter	3pups/sex/litter
Developmental landmarks Pupil constriction PND 21 Sexual maturation	2, 3, 4	 3pups/sex/litter 3pups/sex/litter	 3pups/sex/litter 3pups/sex/litter	 3pups/sex/litter 3pups/sex/litter	 3pups/sex/litter 3pups/sex/litter
Motor activity (PND 14, 18, 22, 58-60)	3	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Auditory startle habituation (PND 23, 59-61)	3	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter

Experimental Parameter	Subgroup	Dose (ppm)			
		0	50	300	500
Passive avoidance (PND 23-25 and retest)	2	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Watermaze (PND 59-63 and retest)	2	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Brain weight					
PND 12	1	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
PND 68-79	4	1♂or♀/litter	1♂or♀/litter	1♂or♀/litter	1♂or♀/litter
Neuropathology					
PND 12	1	1♂or♀/litter	0 [b]	0 [b]	1♂or♀/litter
PND 68-79	4	1♂or♀/litter	0 [b]	0 [b]	1♂or♀/litter
PND 22 [c]	5	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter

a Obtained from page 15 of the study report.
b Examined if treatment-related effects were found at 500 ppm.
c Retained for possible neuropathological evaluation; not examined.
NA Not applicable

5. Dose selection rationale: Dose levels were chosen based on the results of a two-generation reproduction study (MRID 44927702). Rats (30/sex/dose) continuously received dietary concentrations of the test substance at 0, 50, 300 and 600 ppm. According to the study author, treatment-related effects at 300 and 600 ppm included dystocia, decreased pup body weight during lactation, increased liver and thyroid weights, centrilobular hepatocytomegaly, and thyroid follicular cell hypertrophy. Additionally at 600 ppm, decreased body weights in the P females during premating, decreased live-birth index, and lower pup viability index (with cannibalization) in the F1 and F2 generations were observed. The NOEL for this study was 50 ppm. Based on these results, doses of 50, 300, and 500 ppm were chosen for the developmental neurotoxicity study.

6. Dosage administration: All doses were administered to maternal animals continuously in the diet from GD 0 through LD 22.

7. Dosage preparation and analysis: Formulations were prepared by the study sponsor (Bayer Corporation) and shipped to the performing laboratory. Appropriate amounts of test substance in 1% corn oil (w/w) were mixed with diet. Prepared diet mixtures were retained frozen and stored at room temperature during each week of use. Concentrations of the test substance in the diet were evaluated for each formulation used in the study. Prior to the start of the study, stability of the test substance in the diet (20 and 2000 ppm formulations) was evaluated at room temperature and at -20°C (MRID 44927702). Homogeneity of these samples was also evaluated.

Results - Homogeneity Analysis: Results reported in MRID 44927702

6

Stability Analysis: Results reported in MRID 44927702.

Concentration Analysis (range as % of nominal):
50 ppm: 92.4-97.8%
300 ppm: 94.3-99.7%
500 ppm: 95.8-102.2%
Average measured concentrations were 47.6, 289, and 495 ppm for the 50, 300, and 500 ppm dose groups.

The stability and homogeneity analysis data indicated that the general formulation procedures were likely to be acceptable for this study. The concentration analysis data indicated that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: Twice daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals. Gross observations of the dams were conducted daily, prior to treatment. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration. Maternal behavior of the dams was evaluated on lactation days (LD) 1, 5, 8, 14, and 22.

During gestation (daily) and lactation (LD 1, 5, 8, 14, and 22), the dams were observed at approximately the same time each day by an individual who was unaware of each animal's dosage group. The functional observations described below were recorded; however, the study report did not describe the procedures used for these observations, e.g., whether the same technicians were used throughout testing, where the testing was done (no mention was made as to whether animals were observed outside the home cage), when the testing was done with respect to time of dosing, the environmental conditions, whether a scoring or ranking system was used, or the duration of the observation period.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Assessment of lacrimation and salivation, and respiration 2) Presence or absence of piloerection, 3) Observations of urination and/or defecation, 4) Degree of palpebral closure and "prominence of the eye"
X	Incidence of abnormal movements.
X	Incidence of abnormal postures.
X	Incidence of abnormal behavior patterns and/or unusual appearance.

Individual maternal body weight data were recorded daily throughout the exposure period and on the day of sacrifice. In addition, food consumption was recorded daily during the exposure period.

b. Offspring

1) Litter observations: The day of completion of parturition was designated as lactation day (postnatal day, PND) 1. Live pups were counted, sexed and weighed individually for each litter on PNDs 1, 5, 8, 12, 14, 18, and 22. Twice daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity.

On day PND 5, litters were randomly standardized to 10 pups/litter; excess pups were killed and discarded. On PND 12, twenty litters/exposure group were randomly selected for continued examination.

2) Developmental landmarks: Evaluation of pupil constriction was performed once on PND 21 for a total of 3 pups/litter/group for subgroups 2, 3, and 4 (1/sex/litter in each subgroup). Beginning on PND 39, male offspring (a total of 3 pups/sex/litter/exposure group; subgroups 2, 3, and 4) were examined daily for balanopreputial separation. Beginning on postnatal day 28, female offspring (a total of 3 pups/litter/exposure group; subgroups 2, 3, and 4) were examined daily for vaginal patency. The age of onset was recorded.

3) Postweaning observations: Clinical observations were recorded weekly for all animals during the post-weaning period. In addition, rats assigned to subgroups 2 and 3 were examined for gross signs of toxicity when they were weighed or removed from their cages for behavioral testing. Body weights and food consumption were recorded weekly.

4) Neurobehavioral evaluations The offspring subsets were assigned to the following tests. The same animals were used for passive avoidance and water maze testing, and the same animals

were used for motor activity and auditory startle habituation.

i) Functional observational battery (FOB): On PND 12 and weekly during the postweaning period, the offspring in Subset 4 were examined; examination outside the home cage was specified for PND 12 only. Offspring were not subjected to a full FOB; however, observations were made as described above for the dams (assessment of autonomic dysfunction, abnormal posture, abnormal movements, or abnormal behavior patterns).

ii) Motor activity testing: Motor activity was evaluated in 1 pup/sex/litter/exposure group (subset 3) on PNDs 14, 18, 22, and 58-60; the same pups were evaluated each time. A passive infrared sensor mounted outside a stainless-steel 40.6 x 25.4 x 17.8 cm cage (with Plexiglas® flooring during preweaning) was used to record the number of movements and time spent in movement over the course of a 1-hour session, with tabulation at each 10-minute interval. A rack of up to 32 cages and sensors was monitored during each session. Each rat was tested in the same location on the rack across test sessions, and groups were counterbalanced according to sex and treatment level across testing sessions and cages, where possible. No information was provided as to whether testing was performed at the same time of day across sessions.

iii) Auditory startle reflex habituation: Auditory startle reflex habituation testing was performed on 1 pup/sex/litter/exposure group (subset 3) on PNDs 23 and 59-61, using a microcomputer to control the test session. Testing was conducted in a sound-attenuated chamber, using sets of 4 rats per session. Each rat was placed in a small cage above a platform that contained a force transducer in its base. There was an initial adaptation period of 5 minutes, and during the last minute of this period 10 "blank" trials were given to sample the baseline force in the absence of a stimulus. The rats were then given 50 trials of 30 msec, 120 dB bursts of noise at 10-second intervals, followed by an additional 10 "blank" trials. The microcomputer sampled the output of the force transducer and recorded the peak amplitude of each response. The response magnitude was calculated by subtracting the average response on baseline trials, and the average response magnitude and the pattern of responses over 10-trial blocks were compared among treatment groups.

iv) Learning and memory testing: Learning and memory testing was performed on 1 pup/sex/litter/dose group (Subset 2).

Passive avoidance test: A passive avoidance test was conducted on PNDs 23-25 and again seven days later (PNDs 30-32); each animal was tested twice, with a one-week interval between test sessions. For each trial, the animal was placed in the "bright" compartment of a two-compartment chamber, the sliding door between compartments was opened, and the light was turned on. When the animal entered the "dark" compartment, the sliding door was closed, the light was turned off, and a 1 second pulse of 1 mA electric current was delivered to the grid floor of the compartment. The animal was then removed from the apparatus and placed in a holding cage for 30 seconds before the start of the next trial. The criterion for learning was that the rat remain in the "bright" compartment for 60 seconds on two consecutive trials, and trials were repeated until the criterion had been met or until 15 trials had been completed. For each trial the

latency to enter the dark compartment was recorded.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the latency to enter the "dark" compartment on trial 1 of the first test session (activity levels and exploratory tendencies in a new environment); the latency to enter the "dark" compartment on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); and the latency to enter the "dark" compartment on trial 1 of the second session (long-term retention).

Water maze: Water maze testing was conducted on PND 59-63 and again seven days later (PNDs 66-70). Testing was conducted using a watertight, 16-gauge stainless-steel modified M-maze filled with 21±1°C water at a depth of approximately nine inches, and each animal was tested twice, with a one-week interval between test sessions. For each trial, the rat was placed in the starting position at the base of the M-maze, farthest from the two arms and required to swim to one of the two goals to be removed from the water. On the initial trial, the rat had to enter both arms of the maze before being removed from the water, and the first arm chosen was designated as the incorrect goal during the remaining trials of both test sessions. For each trial, the animals were given 60 seconds to make a correct goal choice, and animals failing to make a correct choice within that time were guided to the correct goal and then removed from the water. The inter-trial interval was 15 seconds. The criterion for learning was five consecutive errorless trials, and trials were repeated with a 15-second inter-trial interval until the criterion had been met or until 15 trials had been completed. For each trial, the latency to choose the correct goal and the number of errors, i.e., incorrect turns in the maze, were recorded. No information was provided regarding criteria for scoring errors.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the average number of errors for each trial on the first day of testing (for overall learning performance); the latency to reach the correct goal on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); the average number of errors for each trial in the second session (long-term retention); and the latency to reach the correct goal on trial 1 of the second session (long-term retention).

2. Postmortem observations

a. Maternal animals: Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 25 (rats that did not deliver), LD 12 (dams that delivered a litter that was not selected for continued observation), or LD 22 (dams that delivered a litter that was selected for continued observation) and subjected to gross necropsy of the thoracic, abdominal, and pelvic cavities. The number and distribution of implantation sites was recorded, and abnormal tissues were preserved in 10% neutral buffered formalin for future histopathological examination. Additionally, dams with no surviving pups were sacrificed after the last pup was found dead or missing and presumed cannibalized. Postpartum data for these dams were excluded from summary tables.

b. Offspring: Pups that died prior to litter examinations for pup viability were evaluated for vital status at birth, as previously described. Gross necropsies were conducted on all pups found dead or sacrificed moribund, as well as the pups that were culled on PND 5, the pups from Subset 5 not used as replacement animals (sacrificed on PND 22), and the animals from Subset 4 that were not selected for neuropathological examination (sacrificed on PND 83-87). All pups were sacrificed by carbon dioxide asphyxiation, except for those in Subset 4, which were sacrificed by overdose of sodium pentobarbital. For necropsies conducted on pups dying or sacrificed on or before PND 5, pups with gross lesions were preserved in Bouin's solution. For necropsies conducted after PND 5, gross lesions were preserved in 10% neutral buffered formalin for possible future evaluation. All gross lesions were subjected to histological examination.

The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 12 (subset 1) or 68-79 (subset 4). The reason for the long duration of adult termination ages (i.e., 11 days) was not provided. These animals were subjected to postmortem examinations as described below.

On postnatal day 12, the approximately twenty pups/sex/group of Subset 1 were sacrificed by carbon dioxide asphyxiation and subjected to gross necropsy. The head of each pup was severed just behind the back of the skull and the calvarium was removed from the top of each skull prior to immersion fixation of the entire head in 10% neutral buffered formalin. The heads were then sent to Consultants in Veterinary Pathology, Inc. (Murrysville, PA) for additional processing and evaluation. Upon arrival, the brains were removed and weighed, and 10 undamaged brains/sex/group were randomly selected for microscopic evaluation. Prior to sectioning, the following gross measurements were taken (in a blinded manner) using a Vernier caliper: the anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and the AP length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole. The brains were then cut into six coronal slices approximately 2 mm in thickness, by means of the following cuts: 1) half-way between the ventral base of the olfactory bulbs and the optic chiasm; 2) through the optic chiasm; 3) through the infundibulum; 4) through the midbrain just posterior to the mammillary body; 5) through the cerebellum just anterior to the midpoint; and 6) through the anterior portion of the medulla. The tissues were embedded in paraffin, sectioned at 7 µm, and stained with hematoxylin and eosin, and histopathological examination was performed on tissues from control and high-dose pups. In addition, the following linear microscopic measurements were taken (in a blinded manner), using a calibrated, ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex; 3) diagonal width (maximum cross-sectional width) of the caudate putamen and underlying globus pallidus; 4) thickness of the corpus callosum at its mid point within the section taken at the level of the optic chasim; 5) thickness of the dorsal to lateral portion of the dentate gyrus of the hippocampus within the section taken at the level of the infundibulum (measured bilaterally then averaged; only the mean value was provided in the study report); 6) the maximum height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface; and 7) the thickness of the external germinal layer of the cerebellum (measured at multiple areas over the dorsum of the

11

cerebellum and reported as a mean value only).

On postnatal days 63-79 the 10 animals/sex/group from Subset 4 selected for neurohistological evaluation were sacrificed by administration of heparin and sodium pentobarbital, perfused *in situ* with 10% neutral buffered formalin, and subjected to gross necropsy. The head of each animal was severed between the back of the skull and the first cervical vertebra, and the calvarium was removed from the top of each skull prior to immersion of the entire head in 10% neutral buffered formalin for additional fixation. The dorsal arches of the vertebrae were removed to expose the spinal cord, and the hind limbs were dissected to expose the peripheral nerves. The spinal columns, legs, and heads were sent to Consultants in Veterinary Pathology, Inc. (CVP, Murrysville, PA) for processing and evaluation as follows.

Upon arrival at CVP, the brains were removed and weighed, and 10 brains/sex/group were selected for microscopic evaluation. Prior to sectioning, the following gross measurements were taken (in a blinded manner) using a Vernier caliper: the anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and the AP length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole). The brains were then cut into eleven coronal slices approximately 2-3 mm in thickness, by means of the following cuts: 1) just posterior to the olfactory bulbs; 2) midway between the optic chiasm and the plane of the first section; 3) through the optic chiasm; 4) through the infundibulum; 5) at the posterior edge of the mammillary body; 6) in front of the anterior edge of the pons; 7) just anterior to the middle of the cerebellar cortex; 8) through the posterior portion of the cerebellar cortex; and 9) through the anterior portion of the medulla. The brain tissues and gasserian ganglia and associated trigeminal nerve were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and the Bielschowsky's technique. Histopathological examination was performed on tissues from control and high-dose animals. In addition, the following linear opic measurements were taken (in a blinded manner), using a calibrated, ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex; 3) diagonal width (maximum cross-sectional width) of the caudate putamen and underlying globus pallidus; 4) thickness of the corpus callosum at its mid point within the section taken at the level of the optic chiasm; 5) thickness of the dorsal to lateral portion of the dentate gyrus of the hippocampus within the section taken at the level of the infundibulum (measured bilaterally then averaged; only the mean value was provided in the study report); and the maximum height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface. For those areas measured bilaterally, only the mean was provided in the data report.

The following central and peripheral nervous tissues (X) were dissected, embedded in paraffin (CNS tissues) or glycol methacrylate (PNS tissues), blocked, sectioned, and stained with hematoxylin and eosin, Bielschowsky's technique, and luxol fast blue/cresyl violet (paraffin tissue blocks, 5 micrometer sections) or hematoxylin and eosin, Bielschowsky's technique, and toluidine blue (glycol methacrylate blocks, 2 micrometer sections). Neurohistological evaluation was performed on tissues from males and females in the control and high dose groups.

The following CHECKED (X) tissues were evaluated for adult offspring:

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Olfactory bulbs	X	Sciatic (cross- and longitudinal sections)
X	Cerebral cortex	X	Tibial (cross- and longitudinal sections)
X	Hippocampus	X	Common peroneal (longitudinal section)
X	Basal ganglia	X	Sural (longitudinal section)
X	Thalamus		
X	Hypothalamus		
X	Midbrain		
X	Cerebellum		
X	Pons		
X	Medulla oblongata		
	SPINAL CORD		**OTHER**
	(Cross and longitudinal sections)		
X	Cervical	X	Dorsal root ganglia (longitudinal sections)
X	Thoracic	X	Spinal nerve roots (longitudinal sections)
X	Lumbar		
	OTHER		
X	Gasserian ganglion		
X	Trigeminal nerves		

Data taken from Attachment 4, pp. 525-526, MRID 45516601.

D. DATA ANALYSIS

1. **Statistical analyses:** Data were analyzed by the following statistical procedures:

Parameter	Statistical test

Body weight, food consumption, latency and errors per trial scores in behavioral tests, % mortality per litter	Bartlett's test followed by ANOVA and Dunnett's (parametric) or Kruskal-Wallis and Dunn's (non-parametric)
Variables with graded or count scores, such as litter size, # of trials to criterion in a behavioral test, or day a developmental landmark appears.	Kruskal-Wallis and Dunn's
Clinical observations Other proportion data	Variance test for homogeneity of Binomial Distribution
Motor activity	Repeated measures ANOVA and Dunnett's
>75% of the scores in an exposure group were tied	Fishers Exact test

2. Indices

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

Duration of gestation: Time (in days) elapsed between confirmed mating and the time (in days) the first pup was delivered

Gestation index: (Number of rats with live offspring/number of pregnant rats) x 100

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

Viability index: (Number of live pups on PND 5 (preculling)/number of liveborn pups on PND 1) x 100

Lactation index: (Number of live pups on PND 12 (preculling)/number of live pups on PND 5 (postculling)) x 100

Lactation index: (Number of live pups on PND 22/number of live pups on PND 12 (postculling)) x 100

3. **Historical control data:** Summary historical (negative) control data for motor activity, auditory startle, passive avoidance, water maze, sexual maturation, brain weights, and morphometric measurements were provided.

4. **Positive control data:** Positive control data for neurobehavior and neuropathology were presented in the study report, and are summarized in Attachment 3 to this DER. Most of the positive control studies are unacceptable for use with the current study. None of the studies were conducted within the last few years before the current study. The majority of the studies did not utilize immature rats as test subjects. None of the studies that included motor activity assessment used a 1-hour session with 10-minute blocks. Few of the studies included complete descriptions

of the methods used or tables of individual data. None of the studies demonstrated the laboratory's ability to detect major functional neurotoxic endpoints using the observational methods used in the current study.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** No treatment-related deaths occurred. One control dam was found dead on LD 21. The cause of death could not be determined. One 50 ppm dam was sacrificed on LD 1 because it had no surviving pups (7 stillborn and one cannibalized). Decreased food consumption and body weight were noted toward the end of gestation in this animal. ; The duration of gestation for this dam was 24 days. Clinical signs, such as cold to touch, pale extremities, and localized alopecia, were observed on LD 1.

No treatment-related findings were observed in surviving animals during the conduct of detailed clinical observations. No treatment-related abnormal autonomic functions were reported in any group.

2. **Body weight and food consumption:** Selected group mean body weights, body weight gains, and food consumption values for pregnant or nursing dams are summarized in Table 2. Body weights were decreased ($p \leq 0.05$) in the 500 ppm dams during GDs 2 through 16 (↓4-6%) and in the 300 ppm dams during GDs 2 through 8, 12 and 14 (↓3-4%). Body weights continued to be lower ($p \leq 0.05$) than controls in the 500 ppm dams during LDs 3 through 8 (↓4-6%) and sporadically until weaning (↓5-8%). Incidental decreases ($p \leq 0.05$) in body weights were noted in the 300 ppm dams on LD13 (↓5%) and in the 50 and 300 ppm dams on LD 22 (↓6%). Decreases ($p \leq 0.05$) in maternal body weight gain were noted in the 300 and 500 ppm dams during GDs 0-6 (↓31-56%), and in the 500 ppm dams during LDs 1-4 (↓67%).

Food consumption was decreased ($p \leq 0.01$) in the 300 and 500 ppm dams during GDs 0-6 (↓16-30%) and GDs 0-21 (↓6-13%). Additionally, food consumption was decreased ($p \leq 0.01$) in the 500 ppm dams during GDs 6-15 (↓8-10%) and LDs 4-7 (↓11%). Food efficiency was decreased ($p \leq 0.01$) on GD 0-6 and 0-21 at 300 ppm (↓14% and 4%) and at 500 ppm (↓27% and 9%).

A dose response was evident in the significantly decreased maternal body weight gain and food consumption values reported for early gestation (GD 0-6) at 300 and 500 ppm. Significant, dose-related decreases in GD 0-6 relative food consumption suggest that the effects on body weight gain were not solely due to palatability, but were adverse effects of treatment. During lactation, only the decreased mean body weight gain and food consumption from LD 1-4 at 500 ppm were considered to be treatment-related. All other statistically significant changes were of minimal magnitude (i.e., ≤8%). Decreases of 30-33% in mean body weight gain for all treated groups during the overall lactation period (LD 1-22) were not statistically significant and were not judged to be unequivocally treatment-related.

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

Observations/study week	Dose (ppm)			
	Control	50	300	500
Gestation				
Mean body weight (g)				
Gestation day 0	238.5±10.2	239.3±9.9	238.8±7.0	240.5±10.0
Gestation day 2	246.3±10.5	248.2±11.7	240.1±7.7* (↓3)	236.8±10.1** (↓4)
Gestation day 7	274.8±14.9	275.1±14.6	265.1±11.8* (↓4)	259.2±12.2** (↓6)
Gestation day 21	407.9±23.5	408.2±30.2	404.0±28.6	399.5±22.8
Mean weight gain (g)				
Gestation days 0-6	28.1±7.4	28.8±9.8	19.5±7.2** (↓31)	12.4±10.3** (↓56)
Gestation days 0-21	169.4±19.3	169.2±24.3	165.7±25.8	158.5±20.2
Mean food consumption (g/animal/day)				
Gestation days 0-6	23.0±1.8	23.0±2.4	19.3±2.5** (↓16)	16.1±1.9** (↓30)
Gestation days 9-12	28.3±2.5	28.0±3.2	27.0±2.8	25.9±2.7** (↓8)
Gestation days 0-21	27.1±1.9	27.0±2.4	25.4±2.2** (↓6)	23.7±1.7** (↓13)
Mean relative food consumption (g/kg/day)				
Gestation days 0-6	91.4±5.2	90.5±7.8	78.4±8.6** (↓14)	66.4±7.1** (↓27
Gestation days 0-21	89.5±3.4	89.2±5.3	85.5±4.4** (↓4)	81.6±3.6** (↓9)
Lactation				
Mean body weight (g)				
Lactation day 1	293.8±18.6	295.4±21.1	286.6±16.5	286.2±13.6
Lactation day 6	309.4±19.9	308.8±20.6	303.0±17.5	295.5±17.8* (↓4)
Lactation day 22	350.7±17.6	330.6±31.2* (↓6)	329.1±23.2** (↓6)	324.0±23.0** (↓8)
Mean weight gain (g)				
Lactation days 1-4	13.4±11.0	12.7±14.6	15.5±11.8	4.4±10.8* (↓67)
Lactation days 1-22	57.6±13.5	37.6±35.0 (↓35)	40.0±27.1 (↓31)	38.8±24.8 (↓33)
Mean food consumption (g/animal/day) b				
Lactation days 4-7	52.3±5.0	50.6±7.3	50.2±6.4	46.6±6.7** (↓11)
Lactation days 1-14	51.9±6.0	51.4±4.2	51.0±4.8	50.0±5.6

a Data obtained from Tables B4 through B8 and B10 on pages 91-99 in the study report.
b Maternal food consumption values were not calculated or reported for lactation days 14-22.
n = 20-25
* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

3. **Test Substance Intake:** Based on maternal food consumption, body weight and dietary analyses, 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). [a]

Period	Dose (ppm)		
	50	300	500
Gestation			
Gestation days 0-21	4.4±0.3	25.6±1.3	40.8±1.8
Lactation			
Lactation days 1-14	8.2±0.6	49.4±3.6	82.8±6.7

a Data obtained from Tables B1 and B2, pages 87 and 88 in the study report.

4. **Reproductive performance:** Reproductive performance appeared to be unaffected by the test substance (Table 4).

TABLE 4. Reproductive performance. [a]

Observation	Dose (ppm)			
	0	50	300	500
Number mated	25	25	25	25
Number of litters	24	25	24	25
Intercurrent deaths	1 b	1 c	0	0
Mean (±SD) gestation duration (days)	22.8±0.4	22.6±0.6	22.8±0.4	22.9±0.3
Incidence of dystocia	0	0	0	0

a Data obtained from pages Table B12, page 101 in the study report.
b Died LD 21.
c Killed on LD 1 due to no surviving pups (7 stillborn, and 1 cannibalized).

5. **Maternal postmortem results:** No treatment-related findings were noted at necropsy. Moderate dilation of the kidney pelvis was observed in the 500 ppm dams (2/25 treated vs. 0/25 controls). This finding was considered not to be toxicologically important.

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 5. No treatment-related findings were noted. Mean litter size was not presented after litter standardization on PND 5.

TABLE 5. Litter size and viability. [a]

Observation	Dose (ppm)			
	Control	50	300	500
Total number born	351	349	340	338
Number born live	346	349	336	337
Number stillborn (# litters) [b]	5 (3)	7 (1)	4 (3)	1 (1)
Sex Ratio Day 1 (% ♂)	48.6±14.8	50.8±11.9	50.9±13.2	52.6±13.3
Deaths Days 1-5 [#(%)] [b]	4 (1.2)	5 (1.4)	4 (1.2)	4 (1.2)
Deaths Days 5-12 [#]	0	2	2	2
Deaths Days 14-22 [#]	1	2	2	2
Mean litter size:				
Day 1	14.4±1.9	14.5±2.0	14.0±2.4	13.4±1.8
Day 5 [c]	14.2±1.8	14.4±2.0	13.8±2.2	13.3±1.9
Day 5 [d]	10.0±0.0	10.0±0.0	9.9±0.4	10.0±0.2
Day 11-22	NR	NR	NR	NR
Live birth index	NR	NR	NR	NR
Viability index (%)	98.8	98.6	98.8	98.8
Lactation index (PNDs 5-12)	100	99.2	99.2	99.2
Lactation index (PNDs 12-22)	99.4	98.8	98.7	98.1

a Data obtained from Table B13, pages 102 and 103 in the study report.
b Calculated by the reviewers from data presented in Table B22, pages 143-150.
c Before standardization (culling).
d After standardization (culling).
NR Not reported

Clinical observations in the F1 male 500 ppm animals included misaligned incisors (5/80 treated vs. 0/80 controls) and chromodacryorrhea (5/80 treated vs.0/80 controls); these generally occurred simultaneously in the same animals. Also at 50 and 300 ppm, one male in each group was observed with both malaligned incisors and chromodacryorrhea. The incidences of these findings at 500 ppm were significantly different from controls ($p \leq 0.01$). Although they occurred at relatively low incidences and were not observed until Week 10 postpartum, the possibility that these findings are related to treatment (e.g., a latent expression of a developmental anomaly) cannot be discounted. All other clinical signs noted appeared to be incidental and unrelated to treatment.

2. **Body weight:** Offspring body weights were decreased ($p \leq 0.01$) in both sexes at 300 and 500 ppm on PNDs 8-22 (↓5-15%; Table 6). No other treatment-related differences in pup body weights were noted.

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

Post-natal Day	Dose (ppm)							
	0	50	300	500	0	50	300	500
	Males				Females			
1[d]	7.0±0.52	7.0±0.7	7.2±0.6	7.3±0.6	6.6±0.5	6.6±0.7	6.7±0.5	6.8±0.6
5 [bd]	10.5±0.9	10.6±1.1	10.5±1.3	10.3±0.9	10.2±1.0	10.1±1.2	9.8±1.2	9.7±1.1
5 [c]	10.3±1.1	10.6±1.1	10.4±1.1	10.5±1.1	10.0±1.1	10.0±1.2	9.8±1.0	9.9±1.2
12	23.6±3.0	22.8±3.0	21.2±3.0** (↓10)	20.6±3.1** (↓13)	22.5±3.0	21.7±3.0	20.4±2.5** (↓9)	19.6±3.5** (↓13)
18	32.1±5.5	33.4±3.9	27.8±3.5** (↓13)	27.7±5.5** (↓14)	31.0±5.3	31.8±3.8	26.7±3.8** (↓14)	26.9±5.4** (↓13)
22	43.3±7.1	43.6±6.0	36.8±4.8** (↓15)	37.8±7.7** (↓13)	41.8±7.2	42.0±6.3	35.7±5.3** (↓15)	35.7±7.0** (↓15)

a Data obtained from Tables B23, C5, and C7, pages 151-154, 184, and 186 in the study report. Percent differences from control are presented parenthetically.
b Before standardization (culling).
c After standardization (culling).
d Calculated by the reviewers from individual data presented in Table B23, pages 151 through 154; statistical analyses were not performed.
** Statistically different from control, $p \leq 0.01$

Offspring postweaning body weights were decreased ($p \leq 0.01$) at 300 (↓5-15%) and 500 (↓4-13%) ppm (Table 7). Following the cessation of treatment, there was some recovery of body weight, but mid- and high-dose offspring remained somewhat smaller than controls until termination. Body weight change data (not shown) parallelled this pattern of effect. No other treatment-related differences in body weights were noted.

TABLE 7. Mean (±SD) post-weaning pup body weights (g). [a]

Post-natal Day	Dose (ppm)							
	0	50	300	500	0	50	300	500
	Males				Females			
23	45.8±8.6	47.7±6.8	39.1±5.8** (↓15)	40.2±9.1** (↓12)	44.3±8.3	45.4±7.0	37.5±5.9** (↓15)	38.4±8.5** (↓13)
37	138.4±15.4	139.5±18.5	124.0±14.9** (↓10)	124.0±19.6** (↓10)	121.6±15.3	120.5±13.7	111.2±10.2** (↓9)	111.4±16.1** (↓8)
51	258.0±22.8	261.2±25.0	237.9±22.9** (↓8)	238.6±30.4** (↓8)	184.3±21.3	182.4±17.3	174.3±15.9** (↓5)	176.3±22.4* (↓4)
65	361.0±27.6	364.2±31.8	335.2±32.6** (↓7)	335.5±34.4** (↓7)	234.4±21.9	228.0±21.6	223.0±22.6** (↓5)	227.3±23.1

a Data obtained from pages Tables C5 and C7, pp. 184 and 186 in the study report. Percent differences from control are presented parenthetically. * Statistically different from control, $p \leq 0.05$ ** Statistically different from control, $p \leq 0.01$

3. Developmental landmarks

a) Sexual maturation: Sexual maturation data are presented in Table 8. Increased ($p \leq 0.05$) time to preputial separation was noted in the 300 and 500 ppm male pups when compared to controls (48.2 days each treated vs. 46.7 days controls). In addition, time to vaginal opening was delayed in the 500 ppm females (34.7 days treated vs. 33.4 days controls).

TABLE 8. Mean (±SD) age of sexual maturation (days). [a]

Parameter	Dose (ppm)			
	0	50	300	500
N (M/F)	57/59	59/59	58/60	58/59
Preputial separation (males)	46.7±2.6	47.3±2.5	48.2±2.6**	48.2±3.2*
Vaginal opening (females)	33.4±1.6	33.7±2.0	33.8±1.5	34.7±2.4**

a Data obtained from Table C13, page 192 in the study report.
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

b) Physical landmarks: Pupil constriction was noted in all F1 rats tested on PND 21 (footnotes to Tables C14 and C15, pages 193-233).

4. Behavioral assessments

a) Functional observational battery: Detailed clinical observations revealed no treatment-related effects on autonomic function. It is reiterated that the methods of assessment were not adequately described in the study report.

b) Motor activity: Motor activity data are summarized in Tables 9a (males) and 9b (females) and presented graphically in Attachment 1 to this DER. The mean number of total movements and the mean time spent in movement (not shown) were comparable between treated pups and controls at all assessment intervals. Coefficients of variation were 66-100% for PND 14,41-51% for PND 18 and 21, and 11-14% for adult (PND 58-60) offspring. At each testing session, there were some animals that did not move at all, and some that were continuously moving.

Habituation was not observed in subsession data on PND 14 or 18 (Attachment 1, Figures 4, 6, 8, and 10). For control and 50 ppm groups, the maximum number of movements are always found in the first subsession. For males and females at 300 and 500 ppm the number of movements is increased after the first testing block (15-58%), remaining higher than baseline for 3 or 4 subsessions. No explanation is offered for this finding, which may be related to treatment.

Some habituation was evident in both male and female offspring on PND 22 (Attachment 1, Figures 12 and 14). For PND 62 males and females, habituation was minimal for the number of movements (Attachment 1, Figures 16 and 18). However, subsession data for the time spent in movement demonstrated more definitive habituation in both control and treated groups (Attachment 1, Figures 17 and 19). On all of the testing days, individual motor activity data

demonstrated some animals with little or no habituation.

TABLE 9a. Mean (±S.D.) Number of Movements (counts) during Motor Activity Assessment in F1 Male Pups [a]

Postnatal day/ Subsession		Dose (ppm)			
		0	50	300	500
		Males			
PND 14	1	28.5±28.0	35.0±34.8	21.2±36.2	31.4±35.9
	2	35.8±41.6	28.8±35.0	25.8±38.2	25.6±40.1
	3	45.1±44.6	31.6±36.1	26.4±39.6	21.4±35.1
	4	30.2±38.3	30.8±32.9	29.2±41.5	20.7±32.3
	5	18.8±28.0	31.4±33.4	29.4±36.7	23.0±32.6
	6	20.5±34.6	23.0±31.9	20.8±31.5	29.8±37.4
	Total	178.9±178.6 [100]	180.7±164.9	152.8±191.6	152.0±176.4 (↓15)
PND 18	1	68.0±36.1	70.9±43.0	57.1±38.2	72.4±37.3
	2	69.4±33.8	64.8±47.0	77.6±31.5	76.4±35.1
	3	66.6±43.6	70.8±44.2	84.6±45.1	88.0±35.0 *(↑22)*
	4	62.9±47.0	68.6±55.1	90.5±49.0 *(↑58)*	76.0±45.4
	5	51.2±51.7	64.3±47.5	74.8±53.7	69.6±48.7
	6	45.2±49.2	52.5±59.5	57.6±52.9	61.0±47.3
	Total	363.3±184.3 [51]	391.9±237.9	442.2±183.4	443.5±196.8 (↑22)
PND 23	1	91.0±25.7	93.2 ±23.6	93.6±2.60	82.4±33.6
	2	57.3±40.2	50.9±28.4	69.7±35.8	62.3±41.6
	3	56.3±36.8	53.0±38.3	53.0±37.5	56.8±43.4
	4	53.4±40.9	39.2±32.4	63.9±41.6	60.0±41.0
	5	49.5±49.0	41.4±34.9	61.0±41.9	52.5±46.6
	6	42.7±38.9	44.0±41.5	45.7±38.8	54.4 ±42.6
	Total	350.2±180.3 [51]	321.8±147.8	387.0±177.1	368.5±219.0 (↑5)
PND 58-60	1	135.1±11.8	128.5±12.5	134.0±15.0	129.8±18.2
	2	141.6±14.5	134.7±17.0	142.8±15.5	131.6±25.6
	3	140.1±22.5	133.9±21.1	142.7±15.3	130.0±34.2
	4	127.2±37.11	120.2±20.6	138.7±24.3	131.4±29.8
	5	133.8±26.9	110.2±43.9	132.5±20.6	112.4±51.8
	6	120.3±39.2	116.4±33.3	108.0±43.3	99.7±60.8
	Total	798.1±89.9 [11]	744.0±85.3	798.8±80.7	734.8±168.7 (↑8)

a Data obtained from Table F1, pages 382 through 389 in the study report. Coefficients of variance are presented in brackets. Percent differences from control are presented parenthetically. Within session increases are presented in italicized parentheses.
n = 19-20

TABLE 9a. Mean (±S.D.) Number of Movements (counts) during Motor Activity

Assessment in F1 Female Pups [a]

Postnatal day/ Subsession		Dose (ppm)			
		0	50	300	500
		Females			
PND 14	1	67.8±41.8	43.2 44.06	35.0±38.1	37.6±34.9
	2	59.6±41.7	47.2±43.2	39.8±35.8	47.8±52.5
	3	53.8±44.6	46.0±43.6	32.9±39.3	45.6±58.2
	4	46.4±39.5	44.4±43.9	34.7±36.9	55.4±58.6
	5	37.0±32.0	49.0±48.8	32.6±38.0	48.5±52.4
	6	38.8±43.9	44.4±48.2	33.0±37.2	44.2 ±52.2
	Total	303.6±199.0 [66]	274.4±221.5	207.8±189.8	279.0±285.5 (↓8)
PND 18	1	99.4±36.9	110.8 ±29.16	77.0±31.0	84.0±38.7
	2	93.8±34.6	98.4±45.7	97.8±34.4	89.0±34.2
	3	91.6±41.1	88.3±51.7	100.0±38.8 *(130)*	93.5±39.4
	4	90.2±50.2	95.6±49.3	84.6±48.5	96.4±42.9 *(115)*
	5	82.7±53.8	74.6±55.1	84.2±60.1	90.8±42.2
	6	75.6±60.2	75.2±55.4	68.3±48.2	64.0±53.9
	Total	533.2±217.9 [41]	543.0±242.3	511.8±198.9	517.6±202.0 (↓3)
PND 23	1	105.6±24.2	102.8 ±28.4	96.5±35.7	103.2±27.9
	2	87.1±41.7	71.0±41.3	74.3±35.4	73.0±42.7
	3	60.2±46.1	63.6±48.6	52.6±28.3	62.2±41.7
	4	65.8±43.4	53.5±46.3	60.3±35.9	63.9±36.5
	5	66.9±46.5	51.4±48.8	65.0±42.7	76.2±43.5
	6	59.4±38.7	54.1±49.5	63.4±38.4	69.0 ±42.0
	Total	455.2±194.6 [43]	396.4±219.5	412.0±159.8	447.6±183.9 (↓2)
PND 58-60	1	140.6±11.2	137.2±17.8	139.6±15.2	136.2±12.3
	2	144.4±11.6	147.4±18.6	143.4±14.2	142.2±18.0
	3	150.6±15.7	142.8±12.8	141.9±22.6	142.8±16.9
	4	149.1±23.8	136.6±23.8	145.8±28.6	144.8±20.6
	5	125.8±54.1	112.7±48.3	139.0±25.4	129.5±29.4
	6	113.4±53.8	113.0±48.9	136.6±30.9	114.6±51.0
	Total	823.8±116.9 [14]	789.6±93.5	846.2±103.0	810.0±89.1 (↓2)

a Data obtained from Table F1, pages 382 through 389 in the study report. Coefficients of variance are presented in brackets. Percent differences from control are presented parenthetically. Within session increases are presented in italicized parentheses. n = 19-20

c) **Auditory startle reflex habituation:** Auditory startle data response are summarized in Table 10 and illustrated in Attachment 2 of this DER. Non-significant increases in mean peak amplitude were observed during multiple testing blocks in adult males at 300 and 500 ppm and in adult females at 500 ppm.

Subsession (block) data demonstrated only minimal habituation at PND 23, but indicate evidence of habituation in adult offspring (Attachment 2, Figures 20-23).

TABLE 10. Auditory Startle Reflex Peak Amplitude Data (mean ±S.D.). [a]

Block		Dose (ppm)			
		0	50	300	500
Males					
PND 23	1	10.52±8.22	10.49±6.67	9.07±5.15	10.49±5.60
	2	7.73±5.48	6.90±4.41	6.66±5.28	7.29±4.53
	3	5.95±4.70	5.76±3.89	5.85±4.77	6.68±4.75
	4	6.33±3.81	6.39±3.84	4.90±2.85	6.69±5.39
	5	7.05±4.86	5.86±3.97	5.39±4.02	6.32±6.94
	Mean	7.52±4.72 [63]	7.07±4.13	6.39±3.91	7.49±4.64
	Δ (1-5)	3.47	4.63	3.68	4.17
PND 59-61	1	54.26±36.31	52.30±33.76	72.32±65.73 (↑33)	56.85±33.90
	2	29.88±18.29	32.71±25.02	34.78±32.56 (↑16)	42.84±29.03 (↑43)
	3	19.43±19.46	29.40±22.48	31.94±31.36 (↑64)	29.39±25.78 (↑51)
	4	14.29±10.93	26.35±20.18	23.24±29.12 (↑62)	22.87±18.41 (↑60)
	5	20.59±14.99	25.80±20.99	23.46±22.70 (↑3)	23.66±15.48 (↑15)
	Mean	27.69±15.45 [56]	33.32±21.86 (↑20)	37.14±33.79 (↑34)	35.14±17.10 (↑27)
	Δ (1-5)	33.67	26.50	48.86	33.19
Females					
PND 23	1	10.72±7.18	10.45±8.76	9.01±8.00	12.12±9.17
	2	8.71±5.98	7.58±5.43	6.11±6.37	9.59±8.81
	3	7.75±6.35	7.11±6.29	5.10±3.84	7.96±7.82
	4	8.42±7.78	8.76±7.23	4.86±3.53	10.09±8.77
	5	9.35±9.55	9.95±7.00	6.25±4.53	10.54±11.38
	Mean	8.99±6.76 [75]	8.76±5.82	6.26±4.73	10.06±8.56
	Δ (1-5)	1.37	0.50	2.76	1.58
PND 59-61	1	39.15±24.65	36.08±26.03	34.06±20.89	44.83±30.66 (↑15)
	2	22.12±15.74	19.18±17.98	21.88±21.92	31.28±32.10 (↑41)
	3	17.36±15.04	16.04±16.21	11.74±10.35	20.53±22.74 (↑18)
	4	14.65±10.67	12.62±11.07	17.07±21.71	19.89±24.15 (↑36)
	5	18.21±22.18	12.30±10.25	9.52±8.45 (↓48)	11.47±11.17 (↓37)
	Mean	22.30±15.21 [68]	19.24±13.75	18.86±14.26 (↑15)	25.60±20.90 (↑15)
	Δ (1-5)	20.94	23.78	24.54	33.36

a Data obtained from Table F2, pages 390 and 391 in the study report. Coefficients of variance are presented in brackets. Percent differences from control are presented parenthetically.
n = 16-20

d) Learning and memory testing: The results of passive avoidance and water maze testing for cognitive function are summarized in Tables 11 and 12, respectively. Dose related increases in Trial 2 latency for the first testing session were observed for treated females, statistically significantly for 300 and 500 ppm females ($p \leq 0.05$ and $p \leq 0.01$, respectively) on PND 23-25.

23

A non-significant increase (36%) was seen in males at 500 ppm. Increases in Trial 1 latencies were also seen in this session (57-98%) at 500 ppm demonstrating slower responses prior to the conditioning trial. Although there was no significant decrease in mean latencies for Trial 1/Session2, there was a decrease in all treated groups in the number of rats with latencies of 60 seconds (maximum trial time) on that trial (7-8 controls vs. 1-4 treated), suggesting a decrease in retention for treated rats.

TABLE 11. Passive avoidance performance (mean ± S.D.). [a]

Session/Parameter		Dose (ppm)			
		0	50	300	500
Males					
Session 1 PND 23-25	Trials to criterion	4.3±1.2	5.0±2.8	4.2±1.0	3.9±1.3
	Latency trial 1 (sec)	8.2±7.4	7.8±5.2	11.2±11.1	16.2±15.9
	Latency trial 2 (sec)	32.8±22.1	27.0±22.8	32.9±19.7	44.6±19.5
	Failed to learn	0	1	0	0
Session 2 PND 30-32	Trials to criterion	3.0±0.8	3.0±0.5	3.3±1.1	3.0±0.5
	Latency trial 1 (sec)	31.4±26.3	18.5±13.8	24.4±21.5	18.9±19.4
Females					
Session 1 PND 23-25	Trials to criterion	4.8±1.9	4.1±0.7	4.0±0.9	3.7±1.0
	Latency trial 1 (sec)	10.2±8.7	10.9±9.6	11.0±5.0	16.0±14.5
	Latency trial 2 (sec)	29.2±22.5	33.8±23.3	43.3±21.9*	51.6±14.7**
	Failed to learn	0	0	0	0
Session 2 PND 30-32	Trials to criterion	2.8±0.7	3.1±0.8	3.0±0.7	3.1±0.4
	Latency trial 1 (sec)	27.2±26.2	20.8±20.4	26.6±21.9	23.6±20.0

a Data extracted from Table 363, page 363 of the study report.
n=19-20
NR Not reported
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

No differences from control were observed among treated groups in water maze testing of adult offspring; however, the lack of Session 1/Trial 1 latency data limits evaluation of these data.

TABLE 12. Water maze performance (mean ± S.D.). [a]

Session/Parameter		Dose (ppm)			
		0	50	300	500
Males					
Session 1 PND 59-63	Latency trial 1 (sec)	NR	NR	NR	NR
	Latency trial 2 (sec)	21.6±16.1	13.4±8.7	16.8±8.4	14.3±8.4
	Trials to criterion	9.0±3.0	8.8±2.8	9.8±2.9	9.6±2.6
	Errors per trial	0.49±0.43	0.40±0.26	0.56±0.37	0.44±0.16
	Failed to learn	2	1	2	1
Session 2 PND 66-70	Latency trial 1 (sec)	8.7±4.3	10.9±5.6	11.1±5.8	12.0±9.7
	Trials to criterion	6.0±1.7	7.0±2.4	6.6±2.5	5.7±1.1
	Errors per trial	0.05±0.08	0.13±0.13	0.12±0.15	0.14±0.23
Females					
Session 1 PND 59-63	Latency trial 1 (sec)	NR	NR	NR	NR
	Latency trial 2 (sec)	13.8±7.0	12.8±6.3	12.7±7.8	16.0±9.0
	Trials to criterion	10.8±3.1	8.8±3.0	9.3±2.6	10.6±3.2
	Errors per trial	0.49±0.19	0.36±0.14	0.38±0.15	0.46±0.31
	Failed to learn	1	2	1	3
Session 2 PND 66-70	Latency trial 1 (sec)	14.5±10.8	11.3±4.4	13.4±9.7	14.9±8.0
	Trials to criterion	7.1±2.8	9.3±4.2	8.5±3.7	7.7±2.8
	Errors per trial	0.14±0.14	0.28±0.29	0.22±0.22	0.15±0.17

a Data obtained from Table E2, page 364 in the study report.
n = 16-20; values for rats who failed to learn during session 1 were not included in means for session 2.

5. Postmortem results

a) Brain weights: Mean brain weight data are presented in Table 13. Absolute brain weights were comparable between treated animals and controls on day 12 and at study termination. Increased ($p \leq 0.01$) brain-to-body weight ratios were noted in the males and females on PND 12 (↑10-12%). Decreased ($p \leq 0.01$) terminal body weights were also noted in these animals (↓13-14%).

TABLE 13. Mean (±SD) brain weights. [a]

Parameter	Dose (ppm)			
	0	50	300	500
Males				
Day 12				
Terminal body weight (g)	23.7±3.1	22.5±3.3	21.6±3.2* (↓9)	20.3±3.2** (↓14)
Brain weight (g)	1.31±0.08	1.28±0.08	1.27±0.12	1.27±0.14
Brain-to-body weight ratio (%)	5.640±0.577	5.792±0.632	5.972±0.506	6.314±0.581** (↑12)
Termination (PND 68-79)				
Terminal body weight (g)	420.3±58.8	421.9±37.9	398.1±52.1	381.6±21.1
Brain weight (g)	2.30±0.10	2.25±0.08	2.27±0.12	2.20±0.12
Brain-to-body weight ratio (%)	0.558±0.087	0.535±0.034	0.577±0.071	0.577±0.024
Females				
Day 12				
Terminal body weight (g)	22.4±2.9	21.9±2.9	20.6±2.9	19.4±3.6** (↓13)
Brain weight (g)	1.28±0.08	1.25±0.08	1.26±0.08	1.25±0.14
Brain-to-body weight ratio (%)	5.769±0.553	5.776±0.559	6.112±0.520	6.361±0.550** (↑10)
Termination (PND 68-79)				
Terminal body weight (g)	269.5±35.5	244.9±16.5	253.9±26.2	254.0±28.9
Brain weight (g)	2.12±0.08	2.05±0.12	2.06±0.12	2.00±0.10
Brain-to-body weight ratio (%)	0.796±0.091	0.840±0.082	0.819±0.092	0.794±0.077

a Data obtained from Tables D1 and D2, pages 352 and 353 and Tables G1 and G2 on pages 473 and 474 in the study report.
n =20 on Day 12; n=10 upon study termination
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

b) **Neuropathology**

1) **Macroscopic examination:** No treatment-related findings were observed at necropsy of PND 12 or adult offspring

2) **Microscopic examination:** No treatment-related findings were noted in the qualitative histopathological evaluation of nervous system tissues for PND 12 pups or adult offspring at study termination. No abnormal histopathological findings were observed in PND 12 pups. Findings in treated adult offspring at termination consisted of minimal neuron vacuolation in the dorsal root ganglia of 1/10 males (0/10 control males), minimal myelin sheath swelling of the cervical and thoracic spinal cord in 1/10 females (1/10 controls), and minimal nerve fiber/myelin degeneration of the tibial nerve in 1/10 females (0/10 controls) and of the peroneal/sural nerve in 1/10 females (0/10 controls). The pathologist considered these findings to be within normal background incidences for rats of this age.

3) **Microscopic examination:** Morphometric data are summarized in Table 14. In PND 12 offspring, significant ($p \leq 0.05$) differences from control were observed in the corpus striatum (↓4%) and corpus callosum (↓14%) for 500 ppm males. Additionally, at study termination, the corpus striatum (↓4%) and hippocampal dentate gyrus (↓5%) were significantly smaller ($p \leq 0.05$) in the 500 ppm males as compared to control values. Non-significant decreases in corpus callosum measures were also observed in 500 ppm females at PND 12, and in corpus striatum measures in 500 ppm females at termination. These patterns of response support the interpretation of the findings as being adverse effects of treatment. In accordance with OPPTS 870.6300, further evaluation of brain morphometry at the mid-dose is required, in order to establish a definitive NOAEL for these findings.

Table 14. Mean (±SD) morphometric data for F_1 rats [a]

Parameter	Dose (ppm)							
	0	50	300	500	0	50	300	500
	Males				Females			
PND 12 (subset 1)								
Cerebrum (mm)	12.9±0.35	NA	NA	13.0±0.58	12.8±0.3	NA	NA	12.9±0.31
Cerebellum (mm)	6.0±0.33	NA	NA	6.1±0.50	6.2±0.18	NA	NA	6.1±0.25
Frontal Cortex (µm)	1494±84.6	NA	NA	1521±88.4	1560±80.0	NA	NA	1560±72.1
Parietal Cortex (µm)	1668±77.7	NA	NA	1656±73.2	1701±70.8	NA	NA	1695±72.5
Corpus Striatum (µm)	2286±69.0	NA	NA	2205±94.1* (↓4)	2265±672.5	NA	NA	2253±86.5
Corpus Callosum (µm)	293±37.4	NA	NA	253±44.3 * (↓14)	278±33.9	NA	NA	269±26.8
Hippocampal Gyrus (µm)	1110±44.7	NA	NA	1101±66.4	1137±78.0	NA	NA	1113±86.5
Cerebellum Height (µm)	3840±178.9	NA	NA	3810±251.8	3810±174.9	NA	NA	3882±263.9
Ext. Germinal Layer (µm)	35±4.2	NA	NA	34±3.7	33±2.5	NA	NA	32±2.85
Termination (subset 4)								
Cerebrum (mm)	16.2±0.38	NA	NA	16.3±0.38	16.2±0.52	NA	NA	15.9±0.31
Cerebellum (mm)	8.1±0.30	NA	NA	8.0±0.53	8.0±0.35	NA	NA	7.6±0.45
Frontal Cortex (µm)	1890±78.7	NA	NA	1896±56.2	1791±47.0	NA	NA	1827±42.7
Parietal Cortex (µm)	1929±69.4	NA	NA	1923±62.4	1824±58.0	NA	NA	1869±74.9
Corpus Striatum (µm)	3156±94.7	NA	NA	3018±116.8** (↓4)	3084±110.3	NA	NA	3012±93.0
Corpus Callosum (µm)	304±34.9	NA	NA	302±44.0	256±34.2	NA	NA	255±34.2
Hippocampal Gyrus (µm)	1659±82.5	NA	NA	1578±75.1* (↓5)	1458±55.1	NA	NA	1491±37.6
Cerebellum Height (µm)	5382±333.5	NA	NA	5304±254.9	5214±131.0	NA	NA	5130±200.5

a Data obtained from Appendix O, pages 738 and 788 in the study report.
n=10
NA Not applicable; not measured.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Administration of YRC 2894 (Thiacloprid) in the diet resulted in decreased maternal body weights at 300 and 500 ppm. In the offspring, test substance administration resulted in decreased body weights and body weight gains, delayed sexual maturation, decreased food consumption, decreased terminal body weights on PND 12, and increased relative brain weights on PND 12. No evidence of developmental neurotoxicity was observed. The NOAEL was 50 ppm.

B. REVIEWER COMMENTS

1. PARENTAL ANIMALS: There were no treatment-related effects on maternal survival, clinical or functional observations, reproductive function, or gross pathology at any dietary level. Treatment-related decreases ($p \leq 0.05$) in maternal body weight gain were observed in the 300 and 500 ppm dams during GDs 0-6 (↓31-56%), and in the 500 ppm dams during LDs 1-4 (↓67%).

Treatment-related decreases in food consumption ($p \leq 0.01$) were also noted in the 300 and 500 ppm dams during GDs 0-6 (↓16-30%) and in the 500 ppm dams during LD 4-7 (↓11%). Significant decreases ($p \leq 0.01$) in relative food consumption were also observed on GD 0-6 at 300 (↓14%) and 500 ppm (↓27%), and support the conclusion that the effects on body weight gain in early gestation were not solely related to palatability.

The maternal LOAEL is 300 ppm (25.6 mg/kg/day), based on decreased body weight gain and food consumption during early gestation (GD 0-6). The maternal NOAEL is 50 ppm (4.4 mg/kg/day).

2. Offspring:

Offspring survival, assessments of autonomic function, water maze, brain weights, and qualitative histopathology were unaffected by treatment. Increased incidences ($p \leq 0.01$) of malaligned incisors and chromodacryorrhea were observed at postpartum week 10 in 500 ppm offspring. These findings were considered to be treatment-related, perhaps a latent expression of a developmental anomaly.

As described above, there was suggestive evidence of treatment related changes in motor activity and auditory startle evaluations at 300 and 500 ppm.

Passive avoidance testing revealed significant increases in Trial 2 latency for the first testing session in 300 and 500 ppm females ($p \leq 0.05$ and $p \leq 0.01$, respectively) in weanling offspring. Also at these doses, examination of the individual data indicated some slowing of responses, and suggested an adverse effect on retention of behaviors learned in Session 1.

Pre-weaning body weights were decreased ($p \leq 0.01$) in both sexes at 300 and 500 ppm on PNDs 8-22 (↓5-15%). In addition, postweaning body weights were decreased ($p \leq 0.01$) at 300 (↓5-

15%) and 500 (↓4-13%) ppm. Increased ($p \leq 0.05$) time to preputial separation was noted in the 300 and 500 ppm male pups when compared to controls (48.2 days each treated vs. 46.7 days controls). In addition, time to vaginal opening was delayed in the 500 ppm females (34.7 days treated vs. 33.4 days controls).

The corpus striatum (↓4%) and corpus callosum (↓14%) were smaller ($p \leq 0.05$) in the 500 ppm males when compared to controls on PND 12. Additionally, at study termination, the corpus striatum (↓4%) and dentate gyrus (↓5%) were smaller ($p \leq 0.05$) in the 500 ppm males when compared to controls. Non-significant decreases were also noted in females for corpus callosum measures at PND 12 and for corpus callosum measures at termination, supporting the position that the effects in males are related to treatment. A definitive NOAEL was not established for these findings.

The offspring LOAEL is tentatively set at 300 ppm (25.6 mg/kg/day), based on decreased preweaning and postweaning body weights in both sexes and delayed sexual maturation in the males, and altered performance in passive avoidance testing. The tentative offspring NOAEL is 50 ppm (4.4 mg/kg/day).

This study is classified **acceptable/nonguideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). This study can be upgraded following the submission of acceptable morphometric histopathology data to establish a definitive NOAEL for alterations in brain development, procedural information for functional observation assessments, and adequate positive control data.

C. STUDY DEFICIENCIES:

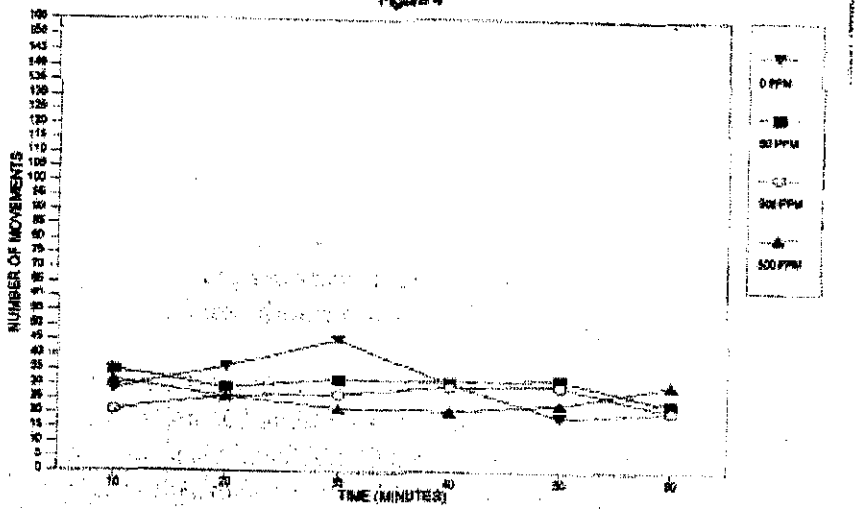
- The method used for the evaluation of functional behavior was not adequately described in the text of the report. The procedures used were not described, including whether the same technicians were used throughout testing, where the testing was done (including whether the animals were removed from the cage), what the environmental conditions were (e.g., noise level, etc.), whether scoring criteria were used for the measured parameters, or the duration of the observation period for open field observations.
- During the period of 10/23/00 to 12/22/00, the humidity in the animal room was outside the targeted range of 30-70% on numerous occasions. There were 7 incidences of >70% humidity, 270 incidences of <30% humidity.
- The lack of habituation of motor activity for the adult rats should be explained.
- Session 1/Trial 1 latency data should be reported for water maze testing. Additionally, more complete reporting of individual learning and memory test results would facilitate the interpretation of these data.
- The termination of adult offspring (subset 4) was conducted on PND 68-79. An explanation for this wide range of ages at termination (i.e., a span of 11 days) should be provided.
- Measurements were made bilaterally for a number of areas of the brain in both PND 12 and adult offspring (see report pages 728 and 779), but only the mean values were reported. Values for linear brain measurements that were recorded, but not included in

the current report, should be submitted.

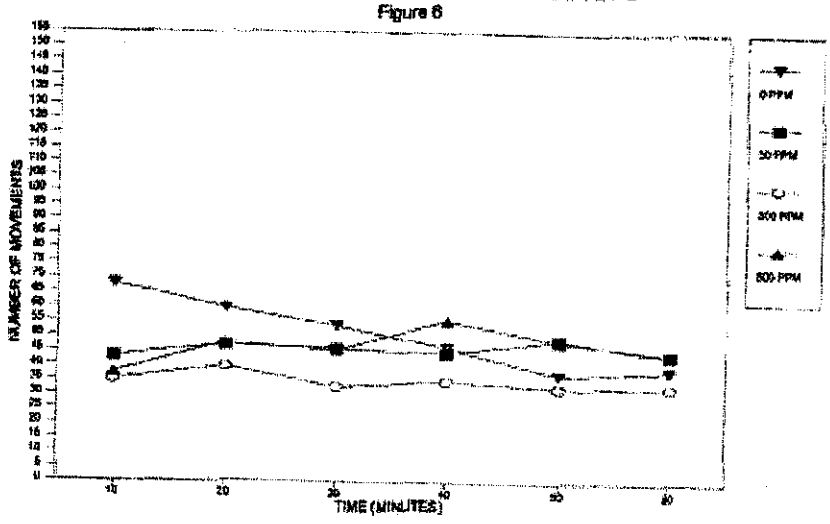
- Treatment-related alterations in brain morphometry were observed at the high-dose (500 ppm). A step-down evaluation of these findings in lower dose group(s) was not performed. Further evaluation of brain morphometry at the mid- and/or low-dose levels is required, in order to establish a definitive NOAEL for treatment-related findings at 500 ppm.
- Acceptable positive control data were not provided.

Attachment 1 - Motor Activity Data

MOTOR ACTIVITY - DAY 14 POSTPARTUM
F1 GENERATION MALE RATS - SUBSET 3
Figure 4



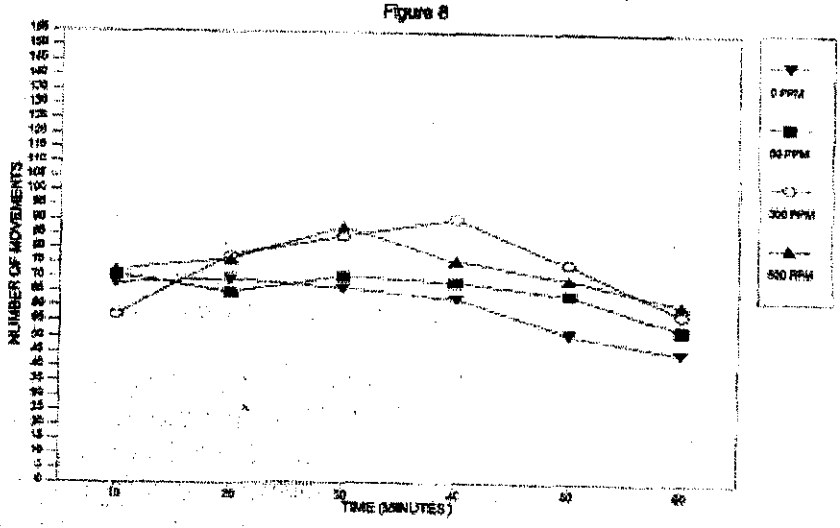
MOTOR ACTIVITY - DAY 14 POSTPARTUM
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 6



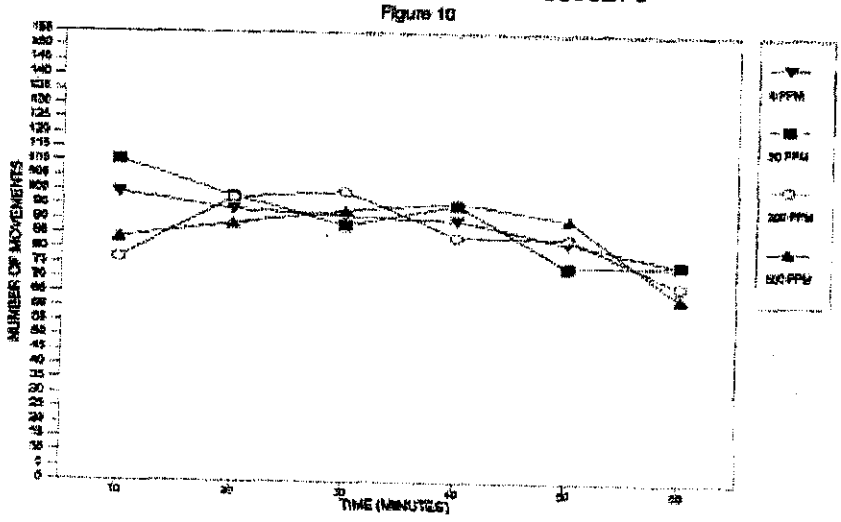
From MRID 45516601, pages 66 and 68.

Attachment 1 - Motor Activity Data - continued

MOTOR ACTIVITY - DAY 18 POSTPARTUM
F1 GENERATION MALE RATS - SUBSET 3
Figure 8



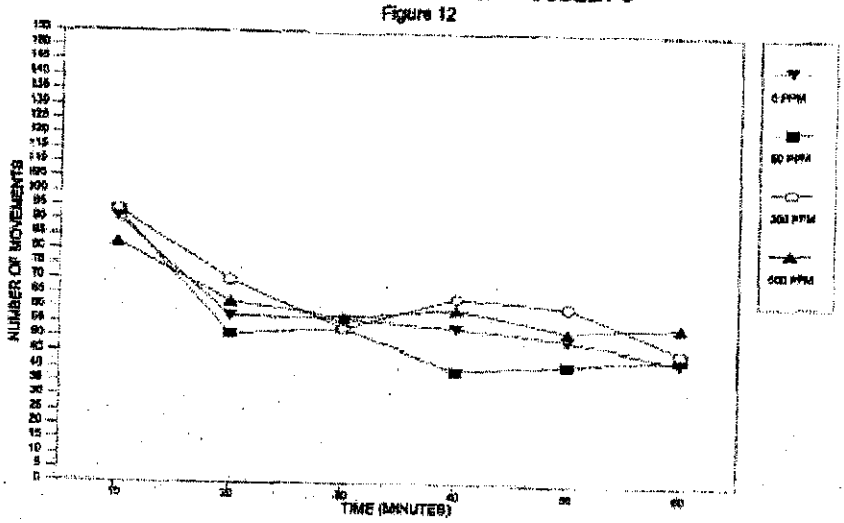
MOTOR ACTIVITY - DAY 18 POSTPARTUM
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 10



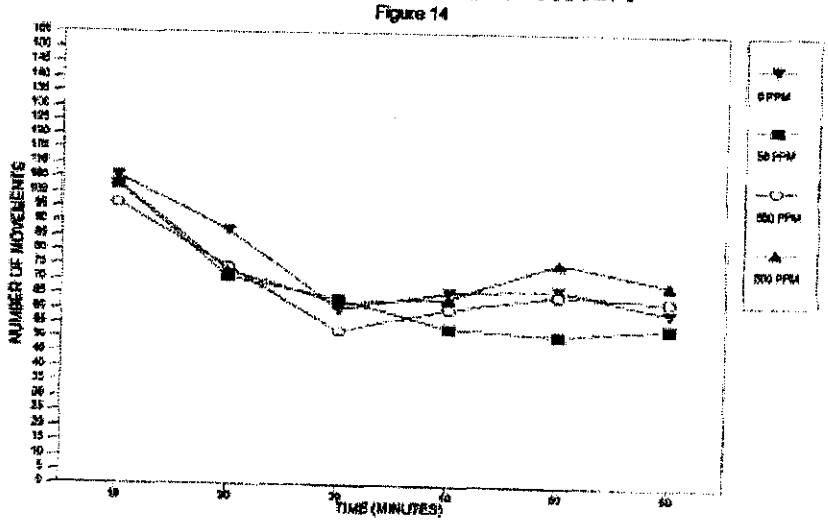
From MRID 45516601, pages 70 and 72.

Attachment 1 - Motor Activity Data - continued

MOTOR ACTIVITY - DAY 22 POSTPARTUM
F1 GENERATION MALE RATS - SUBSET 3
Figure 12



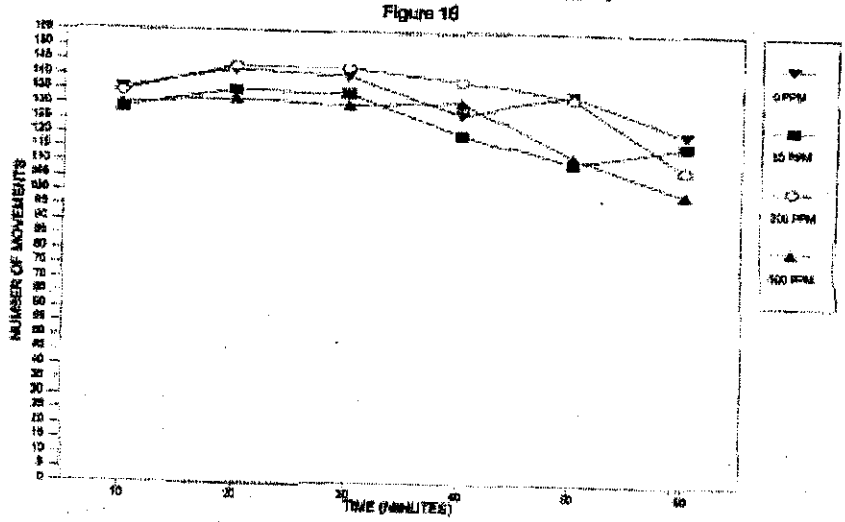
MOTOR ACTIVITY - DAY 22 POSTPARTUM
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 14



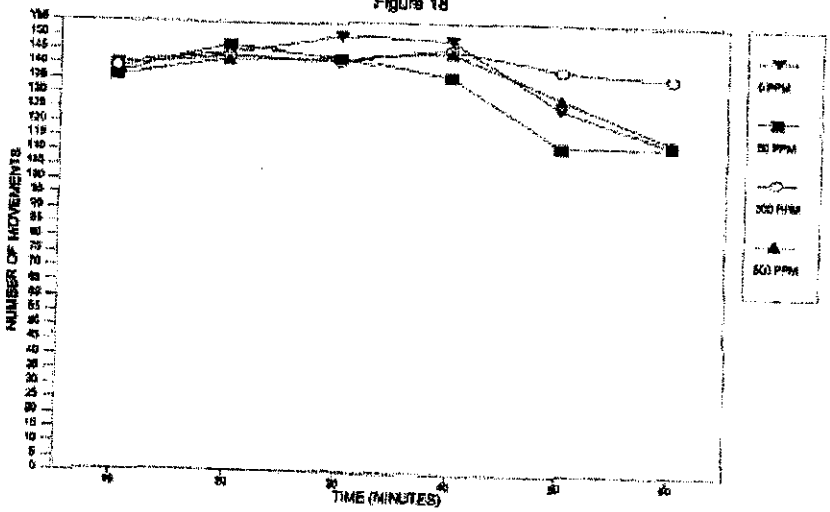
From MRID 45516601, pages 74 and 76.

Attachment 1 - Motor Activity Data - continued

MOTOR ACTIVITY - ADULT
F1 GENERATION MALE RATS - SUBSET 3
Figure 16



MOTOR ACTIVITY - ADULT
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 18

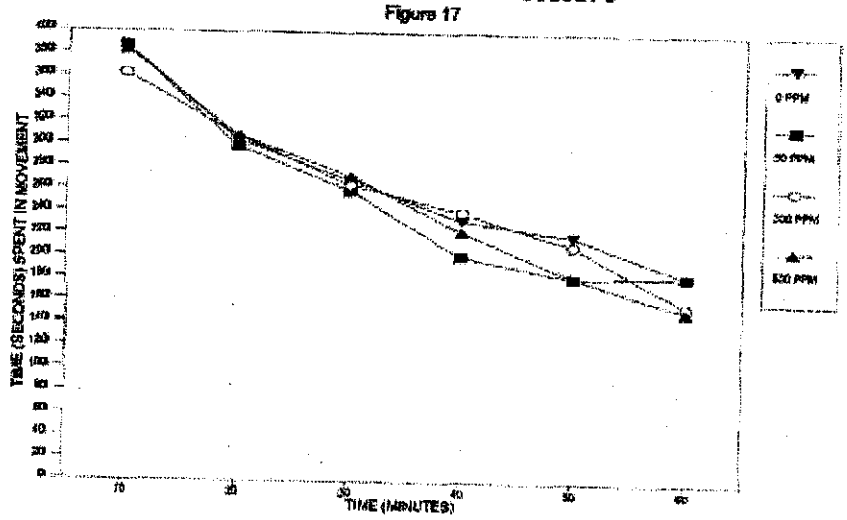


From MRID 45516601, pages 78 and 80.

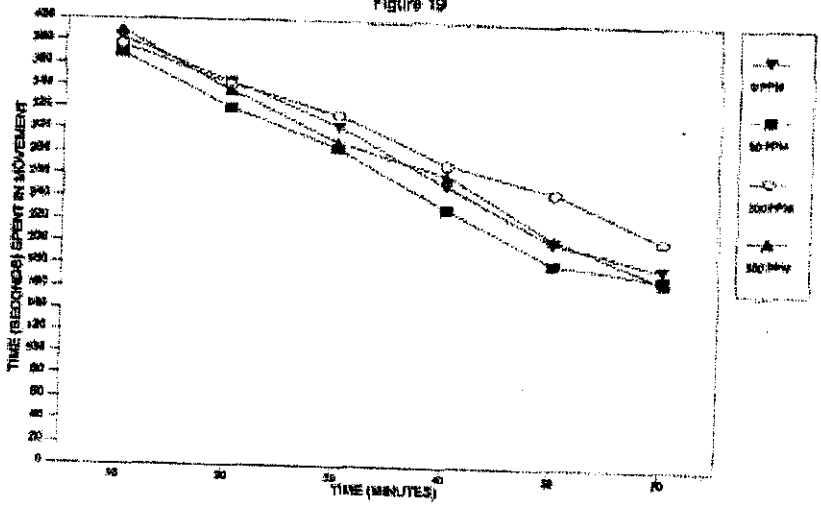
351

Attachment 1 - Motor Activity Data - continued

MOTOR ACTIVITY - ADULT
F1 GENERATION MALE RATS - SUBSET 3
Figure 17



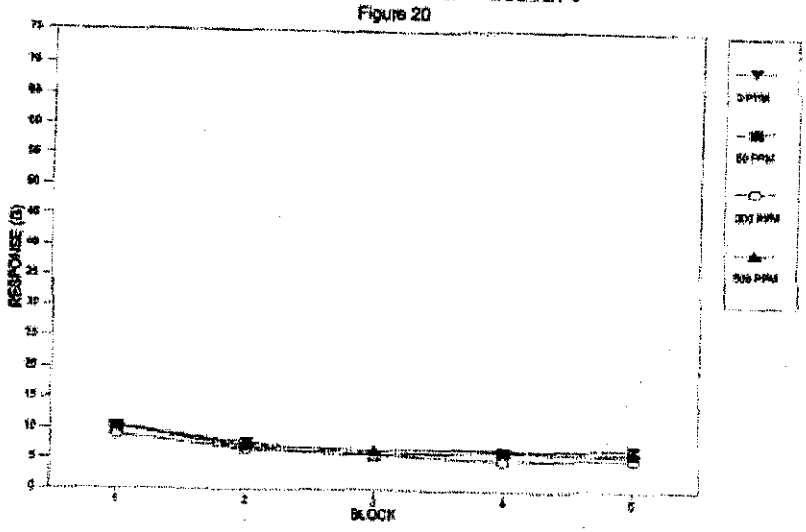
MOTOR ACTIVITY - ADULT
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 19



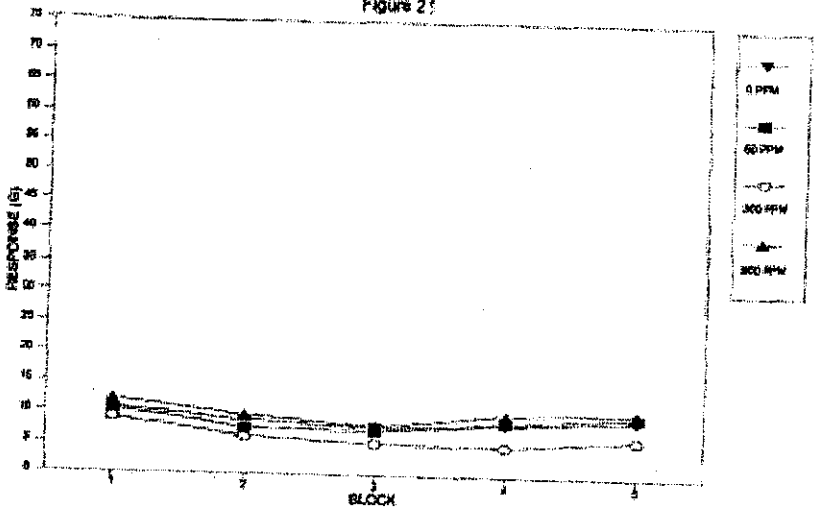
From MRID 45516601, pages 79 and 81.

Attachment 2 - Auditory Startle Habituation Data

AUDITORY STARTLE HABITUATION - DAY 23 POSTPARTUM
F1 GENERATION MALE RATS - SUBSET 3
Figure 20



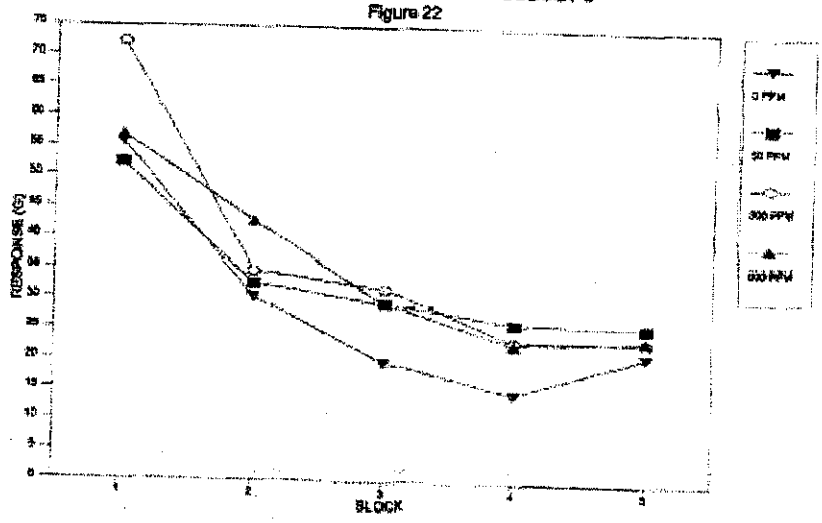
AUDITORY STARTLE HABITUATION - DAY 23 POSTPARTUM
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 21



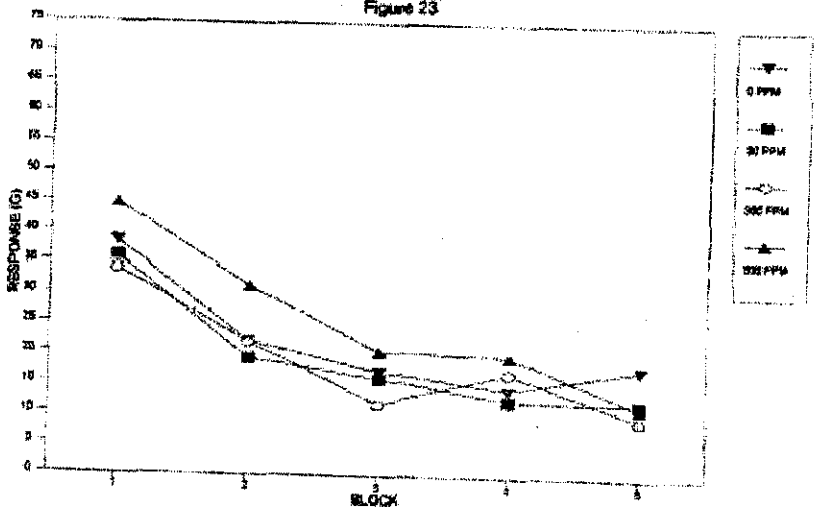
From MRID 45516601, pages 82-83.

Attachment 2 - Auditory Startle Habituation Data - continued

AUDITORY STARTLE HABITUATION - ADULT
F1 GENERATION MALE RATS - SUBSET 3
Figure 22



AUDITORY STARTLE HABITUATION - ADULT
F1 GENERATION FEMALE RATS - SUBSET 3
Figure 23



From MRID 45516601, pages 84-85.

Attachment 3 – Positive Control Data

The following positive control data were provided in the study report (pp.615-714).

Foss, J. and E. Lochry (1991) The assessment of motor activity in neonatal and adult rodents using passive infrared sensors. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 12th annual meeting of the American College of Toxicology; Savannah, Georgia; October, 1991. This study used passive infrared sensors to monitor motor activity of untreated adult rats, untreated adult mice, and neonatal rats on postnatal days 13, 17, 21, and 58-59. The positive control substances d-Amphetamine and chlorpromazine were evaluated in rats at approximately postnatal day 60, and the positive control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon were evaluated in adult rats. Test sessions with positive control substances were 90-115 minutes in duration and comprised of 5-minute blocks.

Foss, J. and E. Riley (1989) Elicitation and modification of the acoustic startle reflex in animals prenatally exposed to cocaine. Journal citation illegible. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study.

Foss, J., E. Lochry, and A. Hoberman (1990) Automated monitoring systems for motor activity and auditory startle applicable for both developmental and adult neurotoxicity studies. Poster presented at the 8th International Neurotoxicity Conference; Little Rock, Arkansas; October, 1990. Motor activity was assessed on postnatal days 13, 17, 21, and 60, using similar equipment to that used in the current study; however, the test session was 1.5 hours long and comprised of 5-minute blocks, while the current study used 1 hour test sessions comprised of 10-minute blocks. Auditory startle habituation was assessed on postnatal days 22 and 60, using similar equipment and methods to those used in the current study.

Lochry, E. and E. Riley (1980) Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. Neurobehavioral Toxicology 2:107-115. This study used different equipment than that used in the current study to assess passive avoidance and learning acquisition and retention and is not acceptable for use as positive control data.

Lochry E., Hoberman A., and M. Christian. (1985) Detection of prenatal effects on learning as a function of differential criteria. Neurobehavioral Toxicology and Teratology 7:697-701. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study.

Lochry, E., J. Foss, and M. Christian (1990) Learning and retention paradigms in developmental neurotoxicity test batteries: passive avoidance and water maze. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 18th European Teratology Society Conference; Edinburgh, Scotland; September 1990. This was a collection of historical control data from passive avoidance and water maze testing conducted in 1988-1989. No further details were provided.

Garman, R.H. (1996) Neuropathology validation report. Consultants in Veterinary Pathology,

P.O. Box 68, Murrysville, PA 15668, Unpublished. Validation included a brief description of the consulting neuropathologist's credentials, experience, and publications.

Garman, R.H. (1993) Neuropathology validation report. Consultants in Veterinary Pathology, P.O. Box 68, Murrysville, PA 15668, Unpublished. Validation included a brief description of the consulting neuropathologist's credentials, experience, and publications, and presented illustrated descriptions of a variety of neuropathological lesions of the central and peripheral nervous systems in adult rats.

Garman R.H. (1996) Neurotoxicity evaluation of positive control substances in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-058. Unpublished. This study used motor activity assessment, auditory startle habituation, and neurohistological examination to evaluate the positive control substances acrylamide, trimethyltin chloride, or MK-801. Motor activity assessment was conducted using similar equipment to that used in the current study; however, sessions were 1.5 hours in duration and comprised of 5 minute blocks, while the current study used 1-hour sessions comprised of 10-minute blocks. Auditory startle habituation testing was conducted using similar equipment and methods as those used in the current study. Similar processing and staining methods were used, and the positive control study evaluated the same brain sections for neuropathology as those evaluated in the F_1 adults in the current study.

Garman, R.H. (1998) Morphometric measurement validation study comparing day 10 and day 12 pups. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Unpublished. This study compared 9 different morphometric measurements between 10 and 12 day old pups. The brains were measured grossly and sectioned similarly to those of the PND 12 pups used in the current study. It was concluded that increases in the thickness of the frontal cortex, height of the cerebellar cortex, and cross-sectional width of the caudate-putamen correlated best with brain maturation between PND 10 and 12. Only the previous two of these three measurements were used in the current study, which also included measurement of the dentate gyrus of the hippocampus.

Foss, J., A. Hoberman, and M. Christian (1992) Developmental neurotoxicity evaluation of lead nitrate in in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the Annual Meeting of the Society of Toxicology; Seattle, Washington; February 1992. Motor activity assessment was conducted using similar equipment to that used in the current study; however, the test session was comprised of 5-minute blocks, while the current study used 10-minute blocks. The equipment and methods used for auditory startle habituation, passive avoidance, and water maze testing were similar to those used in the current study, however no effects of treatment were detected.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
014019	45516601	dev neurotox	rats	GD 0-LD 22	oral	diet	50-500	50, 300, 500 ppm (4.4, 25.6, 40.8 mg/kg/day)	50 ppm = 4.4 mg/kg/day	300 ppm = 25.6 mg/kg/day	decr. BWG, FC	
014019	45516601	dev neurotox	rats	GD 0-LD 22	oral	diet	50-500	50, 300, 500 ppm (4.4, 25.6, 40.8 mg/kg/day)	Tentative: 50 ppm = 4.4 mg/kg/day	Tentative: 300 = 25.6 mg/kg/day	decr. BW, delayed sex. mat. in males, altered passive avoidance performance	Morphomet ric findings at 500 ppm - not examined at 300 ppm