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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

TXR# 0051349

DATE: November 21, 2002

MEMORANDUM:

SUBJECT: **CYMOXANIL** - Review of Developmental Neurotoxicity Study - Rat (MRID 45377901)

PC Nos.:	129106
DP Barcode Nos:	D274848
Submission Nos:	S596594
Tox. Chem. No.:	None

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I. CONCLUSIONS

The Registration Action Branch I has reviewed the developmental neurotoxicity study (MRID 45377901) for cymoxanil. The study is classified as acceptable/non-guideline because of several deficiencies. The study can be upgraded upon submission of additional information to address deficiencies listed below, including: 1) procedural information for functional observation procedures; 2) appropriate positive control data; 3) additional morphometric measurements, as described; 4) additional information regarding motor activity data, as described; 5) additional information regarding temperature excursion; 6) explanation of statistical procedures, as described.

Note: Copy of the DER enclosed.

II. ACTION REQUESTED

On December 2, 1997 (HED Doc. No. 012457, dated 21-JAN-1998), the Health Effects Division Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base for cymoxanil, and assessed the sensitivity of infants and children as required by the 1996 Food Quality Protection Act (FQPA). The HIARC based on the weight-of-the-evidence, recommended that a developmental neurotoxicity study is required for cymoxanil. On August 5, 1999, the HIARC reconfirmed the need for a developmental neurotoxicity study.

III. STUDY REVIEWED

CITATION: York, R. (2001) Oral (gavage) developmental neurotoxicity study of Cymoxanil in Crl:CD®(SD)IGS BR VAF/Plus® presumed pregnant rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 104-021, February 9, 2001. MRID 45377901. Unpublished.

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRID 45377901) Cymoxanil (97.2% a.i.; lot #19062-02) was administered to 25 mated female Crl:CD®(SD)IGS BR VAF/Plus® rats per group in aqueous 0.5% methylcellulose by gavage at dose levels of 0, 5, 50, or 100 mg/kg bw/day from gestation day (GD) 6 through postnatal day (PND) 21. Although the dams were not subjected to a formal functional observational battery (FOB), detailed clinical observations included most of the FOB parameters. 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 24-25 and 31-32) and water maze testing (on PND 59-61 and 66-68) (20/sex/group); 3) motor activity testing (on PND 14, 18, 22, and 60) and auditory startle habituation (on PND 23 and 61) (20/sex/group); 4) detailed clinical exam outside the home cage on PND 12 and weekly during PND 22-79 (20/sex/group), fixed brain weights and neuropathological evaluation on PND 80-83 (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).

Maternal toxicity was limited to slight decreases in body weight (4-6%), body weight gain (17% less than controls for GD 6-21), and food consumption.

The maternal LOAEL is 100 mg/kg/day, based on slight decreases in body weight (4-6%), body weight gain, and food consumption. The maternal NOAEL is 50 mg/kg/day.

At 100 mg/kg/day, there were decreases in pup survival, slight decreases in pup weight during early lactation (less than 6%), increases in morphometric measurements (anterior/posterior cerebrum for males, cerebellar height for females) at PND 79-83, slight decreases in auditory startle reflex amplitude in PND 23 females (61-71% of control levels), decreased performance in passive avoidance in post-weanling animals of both sexes (latency 68-85% of control levels), and decreased retention in the water maze task for adult females (latency 158% of control levels).

At 50 mg/kg/day, there were slight (non-statistically significant) decreases in pup survival, statistically significant decreases in anterior/posterior cerebrum measurements for males, and decreases in retention of the passive avoidance task in weanling animals of both sexes (latency 50-52% of control values).

At 5 mg/kg/day, there were decreases in retention of the passive avoidance task in weanling animals of both sexes (latency 54-75% of control values).

Although substantial changes in motor activity were seen in several groups and time points (for example, decreases up to 52% in PND 18 males, increases up to 129% in PND22 males), no statistically significant changes were seen. The failure to find statistical significance may be attributable to large variance for many values. In addition, motor activity levels for control pups during lactation were substantial below historical control levels. These findings raise questions about the validity of the motor activity assessments during lactation. For adult time points, motor activity was similar for all doses among treated and control animals.

The offspring LOAEL is 5 mg/kg/day, based on decreased retention in the passive avoidance task. The offspring NOAEL is not determined.

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). Classification may be upgradable to guideline upon submission of additional information to address deficiencies listed below, including: 1) procedural information for functional observation procedures; 2) appropriate positive control data; 3) additional morphometric measurements, as described; 4) additional information regarding motor activity data, as described; 5) additional information regarding temperature excursion; 6) explanation of statistical procedures, as described.

DATA EVALUATION RECORD

CYMOXANIL/129106

DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300 (§83-6); OECD 426 (DRAFT)
MRID 45377901

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
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Task Order No. 02-54

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Disclaimer

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

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Date 9/13/2002

Template version 11/01

TXR#: 0051349

KP 09/18/2002

DATA EVALUATION RECORD

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

PC CODE: 129106

DP BARCODE: D274848
SUBMISSION NO.: S596594

TEST MATERIAL (PURITY): Cymoxanil (97.2% a.i.)

SYNONYMS: DPX-T3217; DPX-T3217-113; Curzate; INT-3217; INT-3217-113; 2-Cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino) acetamide

CITATION: York, R. (2001) Oral (gavage) developmental neurotoxicity study of Cymoxanil in Crl:CD®(SD)IGS BR VAF/Plus® presumed pregnant rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 104-021, February 9, 2001. MRID 45377901. Unpublished.

SPONSOR: E.I. Du Pont de Nemours and Company, Wilmington, Delaware 19898.

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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). Classification may be upgradable to guideline upon submission of additional information to address deficiencies listed below, including: 1) procedural information for functional observation procedures; 2) appropriate positive control data; 3) additional morphometric measurements, as described; 4) additional information regarding motor activity data, as described; 5) additional information regarding temperature excursion; 6) explanation of statistical procedures, as described.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Cymoxanil (T3217 Technical)

Description:	a pink powder
Lot/Batch #:	19062-02 (listed in the study report, but not on the Certificate of Analysis)
Purity:	97.2% a.i.
Compound Stability:	not reported (stored refrigerated)
CAS # of TGAI:	57966-95-7

2. Vehicle and/or positive control: The vehicle was aqueous 0.5% methylcellulose, prepared with methylcellulose (Lot number 77H1079) in reverse osmosis membrane processed deionized water.

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®	
Age at study initiation:	Approximately 11 wks	
Wt. at study initiation:	226-256 g	
Source:	Charles River Laboratories, Inc., St. Constant, Quebec, Canada	
Housing:	Individually in stainless-steel wire-bottomed cages except during cohabitation, when animals were housed in pairs, and late gestation/postpartum, when animals were housed in nesting boxes.	
Diet:	Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, MO) *ad libitum*	
Water:	Local water (purified via reverse osmosis and chlorinated) *ad libitum*	
Environmental conditions:	**Temperature:**	65-70 °F (note one excursion to 100°F on April 13-14)
	Humidity:	43-59% (note one excursion to 75% on April 13-14)
	Air changes:	at least 10/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	up to 7 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start of dosing: January 19, 2000 (GD0: January 13, 2000); End: April 28, 2000

2. Study schedule: Females were mated and assigned to groups of 25 animals. The test substance was administered from gestation day (GD) 6 through either postnatal day (PND) 21 for dams that delivered a litter or presumptive GD 24 for females that did not deliver a litter. The day of birth was designated as PND 1 or lactation day (LD) 1. Pups were weaned on PND 22, after which time maternal animals were killed. F_1 animals remained on study up to approximately PND 83.

3. Mating procedure: Females were paired 1:1 with males of the same strain and source for a maximum of 7 days. Females were 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 as GD 0, and the female was returned to individual housing. Pregnant

females were housed in nesting boxes from GD 20 through LD 22.

4. Animal Assignment: Mated females were assigned to groups, using a computer-generated randomization procedure based on GD 0 body weights (Table 1). Dams were assigned to functional observation testing as described below.

On PND 12, offspring were assigned to testing Subsets 1-5, using a table of random units in such a way that whenever possible each litter had one male and one female pup assigned to each subset, all litters were represented, and an equal number of male and female pups was selected from each dose level. A table of random units was also used to select 10 offspring/sex from Subsets 1 and 4 for neurohistological examination. The testing conducted and the subsets used for each test are given in Table 1. Pups from Subset 5 were retained from PND 12-22 for potential use as replacement animals and to standardize litter size (to 8 pups/litter).

Table 1. Study design

Experimental Parameter/Pup subset		Dose (mg/kg/day)			
		0	5	50	100
Maternal Animals					
No. of maternal animals assigned		25	25	25	25
Offspring					
Detailed clinical exam					
PND 5	[Random]	10/sex	10/sex	10/sex	10/sex
PND 12, weekly during PND 22-79	[Subset 4]	20/sex	20/sex	20/sex	20/sex
Developmental milestones/sexual maturity	[Subsets 2-4]	60/sex	60/sex	60/sex	60/sex
Motor activity (PND 14, 18, 22, 60)	[Subset 3]	20/sex	20/sex	20/sex	20/sex
Auditory startle habituation (PND 23, 61)	[Subset 3]	20/sex	20/sex	20/sex	20/sex
Passive avoidance (PND 24-25, 31-32)	[Subset 2]	20/sex	20/sex	20/sex	20/sex
Water maze testing (PND 59-61, 66-68)	[Subset 2]	20/sex	20/sex	20/sex	20/sex
Brain weight (fixed)					
PND 12	[Subset 1]	20/sex	20/sex	20/sex	20/sex
PND 80-83	[Subset 4]	10/sex	10/sex	10/sex	10/sex
Neuropathology					
PND 12	[Subset 1]	10/sex	10/sex	10/sex	10/sex
PND 80-83	[Subset 4]	10/sex	10/sex	10/sex	10/sex

Data taken from text table, p. 30, and text, pp. 34-39, MRID 45377901.

5. Dose selection rationale: Dose levels were chosen based on the results of a developmental toxicity study (MRID 43616524) in which Cymoxanil was administered to 25 presumed pregnant female rats per group in 0.5% (w/v) methylcellulose by gavage at dose levels of 0, 10,

25, 75, or 150 mg/kg bw/day from gestation days 7 through 16. According to the study report, maternal toxicity was evident at 25 mg/kg bw/day as decreased maternal weight gain during GD 7-9, while the 75 and 150 mg/kg bw/day groups had decreased body weight gains throughout dosing. Developmental toxicity was evident at 25, 75, and 150 mg/kg bw/day as dose-dependent increases in developmental variations which were generally consistent with delayed ossification. At 150 mg/kg bw/day, the mean fetal weight and number of live fetuses were significantly decreased, and the number of resorptions per litter was signficantly increased. Dose levels of 5, 50, and 100 mg/kg bw/day were selected for the current study with the expectation that 5 mg/kg bw/day would be a NOEL or minimally effective dose level for developmental neurotoxicity and that 100 mg/kg bw/day would have adverse developmental effects without causing excessive pup mortality.

6. Dosage administration: All doses were administered once daily to maternal animals by gavage, on GD 6 through PND 21 or presumptive GD 24, in a volume of 10 mL/kg of body weight/day. Dosing was based on the most recent body weight determination.

7. Dosage preparation and analysis

Formulations were prepared daily by mixing appropriate amounts of test substance with 0.5% (w/v) methylcellulose and were stored refrigerated. Prior to sampling or dose administration, the formulations were stirred with a magnetic stir bar and a stir plate for at least one hour. On the first day of preparation two sets of duplicate samples of all dosing formulations were taken for stability evaluation; one set was frozen immediately, and the other set was frozen after retention at room temperature for 5 hours. Duplicate samples were taken from the top, middle, and bottom of all dosing formulations on the first day of dosing for homogeneity and concentration analysis. Samples of the dosing formulations for all treatment levels were analyzed for concentration on two other occasions: once at the approximate middle of the study and once at the end of the study.

Results - Homogeneity Analysis: The mean concentrations of the samples from the low-, mid-, and high-dose formulations were 96.2, 96.4, and 99.5% of nominal, respectively, and the measured concentrations of all individual subsamples ranged from 93.0-100.2% of nominal.

Stability Analysis: Concentrations of samples from the low-, mid-, and high-dose formulations held at room temperature for 5 hours were 96.0, 96.4, and 97.7% of nominal, respectively. Concentrations of samples from the low-, mid-, and high-dose formulations stored frozen for 3 days were 97.6, 95.4, and 101.0% of nominal, respectively. Concentrations of samples from the low-, mid-, and high-dose formulations stored frozen for 8 days were 99.4, 92.2, and 99.0% of nominal.

Concentration Analysis: Absence of the test material was confirmed in the vehicle. Concentrations of samples taken at the middle and end of the study from the low-, mid-, and high-dose formulations were 96.4-100.6, 97.0-102.4, and 93.8-97.6% of nominal, respectively.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: All animals were checked twice daily for mortality or moribundity and at least once daily during dosing for clinical signs of toxicity. Maternal behavior of the dams was evaluated on PNDs 1, 5, 8, 14, and 22.

Dams were not subjected to a full functional observational battery (FOB). However, during treatment (GD 6 through PND 21), 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.

Data taken from text, p. 34, MRID 45377901.

Individual maternal body weights were recorded on GD 0, daily during the dosing and post-dosing intervals, and at termination. Food consumption was recorded on GD 0 and daily during the dosing and post-dosing interval; however, food consumption values were not reported after PND 14, when the pups presumably began to consume maternal feed.

b. Offspring:

1) Litter observations: The day of completion of parturition was designated as lactation day (postnatal day) 1. Litter size, live litter size, and pup viability at birth were evaluated, and for pups that died prior to the initial viability examination, the lungs were removed and immersed in

water to determine whether the pup was live- or stillborn. Litters were checked for dead pups at least twice daily, and numbers of pups in each litter and clinical observations were recorded once daily preweaning. Live pups were weighed individually on PND 1, 5, 8, 12, 14, 18, and 22.

On PND 5, litters were standardized using a table of random units to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible), and the excess pups were killed and necropsied. Litters with fewer than 9 pups were retained until PND 12, at which time the offspring were assigned to Subsets 1-5, as previously described.

2) Developmental landmarks: Beginning on PND 28, female offspring were examined daily for vaginal patency. Beginning on PND 39, male offspring were examined daily for balanopreputial separation.

3) Postweaning observations: After weaning on PND 22, offspring were examined at least twice daily for mortality or moribundity, and clinical observations and individual body weights were recorded once weekly. Food consumption was recorded weekly beginning on PND 30.

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 5, 10 randomly selected pups/sex/group (1/sex/litter from 10 litters) were examined outside the home cage (no information regarding performance of this examination was provided). 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. Rats assigned to Subsets 2 and 3 were "examined for gross signs of toxicity" when they were removed from their cages for behavioral testing and/or weighed.

ii) Motor activity testing: Motor activity was evaluated in the pups from Subset 3 on PND 14, 18, 22, and 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 evaluated in the pups from Subset 3 on PND 23 and 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 the offspring from Subset 2.

(1) Passive avoidance testing: A passive avoidance test was conducted on PNDs 24 or 25 and 31 or 32 to assess learning, short-term retention, long-term retention, and hyperactivity; 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 remained 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).

(2) Water maze testing: Water maze testing was conducted on PND 59-61 and 66-68 to assess overt coordination, swimming ability, learning, and memory. 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 PND 22 and subjected to gross necropsy of the thoracic, abdominal, and pelvic cavities, and the number and distribution of each dam's implantation sites were recorded. Females that delivered, but had no surviving pups were sacrificed after the death of the last pup, and subjected to gross necropsy. Females that did not deliver a litter were sacrificed on GD 25 and examined for gross lesions; grossly nongravid uteri were examined while being pressed between glass plates to confirm the absence of implantation sites and retained in 10% neutral buffered formalin.

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 79-83). 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 fixed brain weight and neuropathological evaluation were sacrificed on PND 12 or on PND 79-83. 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 edge of the cerebellar uvula (this measurement was taken on the diagonal). 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) just anterior to the optic chiasm; 3) through the median eminence just anterior to the infundibulum; 4) through the midbrain at the posterior edge of the mammillary body; 5) through the cerebellum just anterior to its midpoint; and 6) through the anterior portion of the medulla. The tissues were embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin, and histopathological examination was performed on tissues from control and high-dose pups. In addition, the following 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 dorsal 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 3) 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.

On postnatal days 79-83, 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 column and legs were placed in neutral buffered formalin for additional fixation and sent to Experimental Pathology Laboratories, Inc. (Herndon, VA) for processing and examination. The 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 (uvula) (this measurement was taken on the diagonal). 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) just anterior to the optic chiasm; 4) through the median eminence just anterior to the infundibulum; 5) just anterior to the posterior edge of the mammillary body; 6) immediately 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 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 (measured bilaterally then averaged); 2) thickness of the dorsal portion of the dentate gyrus of the

hippocampus within the section taken at the level of the infundibulum (measured bilaterally then averaged); and 3) 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, preserved 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 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 Appendix A, pp. 507-510, and Table 3, pp. 516-520, respectively, MRID 45377901.

D. DATA ANALYSIS

1. **Statistical analyses:** Interval or ratio data, including body weights, food consumption, latency

and errors per trial in behavioral tests, and percent mortality per litter were first analyzed using Bartlett's test of homogeneity of variances at a $p<0.001$ significance level. Homogeneous data were then analyzed using ANOVA at a $p<0.001$ significance level, followed by Dunnett's test if significant. Data found to have nonhomogeneous variances using Bartlett's test and data that had graded or count scores, including litter size, the number of trials to criterion in a behavioral test, and the day a developmental landmark was noted, were analyzed using nonparametric methods. If 75% or fewer of the scores in all dose groups were tied, the data were analyzed using the Kruskal-Wallis Test, followed by Dunn's test if significant. If more than 75% of the scores in all dose groups were tied, the proportions of ties in each group were compared using Fisher's Exact Test. Incidences of clinical observations and other proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Data with measurements recorded at intervals throughout each test session, including motor activity and auditory startle habituation tests, were analyzed using Analysis of Variance with Repeated Measures. If the Dosage effect was significant, totals were compared using Dunnett's test, and if the Dosage x Block interaction was significant, the data at each measurement period were analyzed using ANOVA, followed by Dunnett's test if significant. Significance levels of $p<0.05$ or $p<0.01$ were used for all tests except those already mentioned as using $p<0.001$.

2. Indices:

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

Gestation index = (Number of animals with live offspring/number of pregnant animals) x 100

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

Viability index (%) = (# live pups on PND 5 precull/# live pups on PND 1) x 100

LD 5-12 Lactation index (%) = (# live pups on PND 12/# live pups on PND 5 postcull) x 100

LD 12-22 Lactation index (%) = (# live pups on PND 22/# live pups on PND 12) x 100, where the number of live pups on PND 22 excludes those not selected for further evaluation, and the number of live pups on PND 12 excludes those sacrificed for fixed brain weights and/or neurohistological evaluations.

3. Positive and historical control data:

A very large quantity of "positive control data" were included in the study report (see Appendix for detailed summary); however, most of it is unacceptable for use with the current study. None of the studies were conducted within the last few years before the current study. 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.

Historical control data were also provided for FOB, developmental landmarks, motor activity, passive avoidance, auditory startle, wtaer maze, brain weights, and morphometrics (pp. 654-751).

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical and functional observations: One mid-dose female was found dead 2 hours and 55 minutes post dosing on PND 21, with no prior abnormal clinical signs and no abnormal gross necropsy findings, and the death was presumed to be due to an intubation-related injury. Two high-dose females exhibited hyperreactivity on the first day of dosing, and one high-dose female exhibited excess salivation on GD 16, although her record indicated that her "autonomic functions appeared normal" on that day. Findings that were observed at low incidences in treated and control groups and/or without a dose-response pattern included the following: chromorhinorrhea, localized alopecia, bent tails, a mass, scab, or ulceration, dehydration, and emaciation. Separate tables listing 'FOB' findings were not provided, and results of these observations were not separately discussed in the report.

2. Body weight and food consumption: Selected group mean body weights and food consumption data for pregnant or nursing dams are given in Table 2. High-dose females had a mean decrease in body weight during the GD 6-9 interval and had a decreased mean body weight gain for the GD 6-21 interval (83% of control, $p<0.01$) followed by increased cumulative body weight gain during LD 1-22 (135% of control, $p<.05$), as compared to controls. The mean absolute body weights of the high-dose group were statistically significantly decreased ($p<0.05$ or $p<0.01$) from GD 7-21, and on LD 2-7; however, the body weights were only 4-6% less than controls.

Mid-dose females had decreased body weight gain during GD 6-9 (51% of controls, $p<0.01$), however the absolute difference in body weight between mid-dose and control animals was only 3.4 g (n.s.), and this difference in body weight gain is not considered biologically significant at the mid-dose level. Mid-dose females had increased cumulative body weight gain during LD 1-22 (113% of controls, n.s.).

Mean food consumption of the high-dose group was decreased during GD 6-15 and 18-21 (74-90% and 85% of controls, respectively; $p<0.01$), while mean food consumption of the mid-dose group was decreased only during GD 6-12 (87-93% of controls, $p<0.05$).

TABLE 2. Selected mean maternal body weight and food consumption data [a]

Observations/study interval	Dose (mg/kg/day)			
	Control	5	50	100
Gestation [b]				
Mean body weight (g)				
Gestation day 0	241.1±7.9	240.6±7.9	241.2±7.9	241.0±7.7
Gestation day 6	268.4±10.6	266.8±10.2	270.6±10.9	266.6±8.8
Gestation day 9	279.8±11.6	278.8±11.5	276.4±13.0	266.2±10.4 (95) [c] **
Gestation day 15	313.6±13.0	313.4±16.3	310.0±15.9	299.4±15.1 (95) **
Gestation day 21	390.3±16.4	389.2±23.0	388.5±20.2	368.2±21.9 (94) **
Mean weight gain (g)				
Gestation days 0-6	27.3±7.0	26.2±6.4	29.4±8.2	25.6±5.3
Gestation days 6-9	11.4±3.7	12.0±3.9	5.8±4.9 (51) **	-0.4±6.4 **
Gestation days 6-21 [d]	122.2±12.1	121.7±15.5	117.9±14.8	101.7±18.7 (83) **
Mean food consumption (g/animal/day)				
Gestation days 0-6	22.1±1.9	21.9±2.0	22.4±1.8	22.6±2.1
Gestation days 6-21 [d]	24.4±2.0	24.1±1.9	23.2±2.1	21.2±2.1 (87) **
Lactation				
Mean body weight (g)				
Lactation day 1 [e]	282.2±14.2	278.5±17.6	281.1±16.6	270.2±17.8
Lactation day 6 [f]	301.4±14.4	291.1±16.5 (97) *	297.1±17.8	284.0±18.0 (94) **
Lactation day 12 [g]	321.4±17.3	317.6±17.6	316.5±18.9	309.4±19.1
Lactation day 18 [h]	328.2±17.3	326.5±17.5	329.0±15.3	321.5±20.3
Lactation day 22 [i]	320.8±16.7	321.8±20.6	328.3±22.1	327.0±24.2
Mean weight gain (g)				
Lactation days 1-22 [j]	41.2±13.1	39.0±19.2	46.6±17.8 (113)	55.8±18.9 (135) *
Mean food consumption (g/animal/day)				
Lactation days 1-14 [h]	46.7±5.2	46.2±4.6	47.0±4.2	43.5±3.5 (93) *

Data taken from Tables B2-B6 and B8, pp. 60-66 and 68, respectively, MRID 45377901.

a Data are given as Mean ± S.D.

b Unless otherwise noted, N = 25, 25, 22, and 24 for the control, low-, mid-, and high-dose groups, respectively.

c Numbers in parentheses equal percent of control; calculated by reviewer.

d N = 21, 22, 22, 24 for the control, low-, mid-, and high-dose groups, respectively; excludes dams that delivered.

e N = 25, 24, 22, 24 for the control, low-, mid-, and high-dose groups, respectively; excludes erroneous values and/or those associated with interrupted water access.

f N = 25, 25, 22, 22 for the control, low-, mid-, and high-dose groups, respectively; excludes high-dose dams that had no surviving pups.

g N = 24, 22, 22, 22 for the control, low-, mid-, and high-dose groups, respectively; excludes erroneous values and/or those associated with interrupted water access and high-dose dams that had no surviving pups.

h N = 20, 20, 20, 21 for the control, low-, mid-, and high-dose groups, respectively; excludes dams not selected for further observation and high-dose dams that had no surviving pups.

i N = 20, 20, 19, 20 for the control, low-, mid-, and high-dose groups, respectively; excludes dams not selected for further observation and a mid-dose dam that died on LD 21.

j N = 20, 19, 19, 20 for the control, low-, mid-, and high-dose groups, respectively; excludes erroneous values, dams not selected for further observation, and a mid-dose dam that died on LD 21.

Significantly different from control: * $p<0.05$; ** $p<0.01$.

3. Reproductive performance: Results for the maternal animals are summarized in Table 3. The pregnancy rates, gestation indices, length of gestation, and numbers of implantations per dam of the treated groups were similar to controls. Two high-dose dams had whole litter losses by LD 5.

TABLE 3. Reproductive Performance

Observation	Dose (mg/kg/day)			
	Control	5	50	100
Number mated	25	25	25	25
Number (%) [a] pregnant	25 (100)	25 (100)	22 (88)	24 (96)
Number of litters	25	25	22	24
Intercurrent deaths	0	0	1	0
Gestation index (%)	100.0	100.0	100.0	100.0
Mean (±SD) gestation duration (days)	22.7±0.5	22.6±0.5	23.0±0.4	23.0±0.2
Mean (±SD) implantation sites/dam	16.2±1.3	15.8±1.8	16.1±1.6	16.4±1.4
Incidence of dystocia	0	0	0	0
Number (%) of dams with all pups dying LD 1-4	0	0	0	2 (8.3)
Number (%) of dams with all pups dying LD 5-22	0	0	0	0

Data taken from Table B10, p. 70, MRID 45377901.
a Calculated by reviewer.

5. Maternal postmortem results: There were no treatment-related abnormal necropsy findings. Gross lesions were limited to confirmation of previously noted abnormal clinical signs, including localized alopecia, bent tails, a scab on one animal's neck, and one high-dose female with emaciation/dehydration.

B. OFFSPRING

1. Viability and clinical signs: Litter size and viability (survival) results from pups during lactation are summarized in Table 4. There were no treatment-related effects on numbers of litters, live litter size, or sex ratios at birth. The high-dose group had increased numbers of pups found dead or presumed cannibalized throughout lactation, significantly decreased viability index and lactation indices (for both LD 5-12 and 12-22), and significantly decreased mean litter sizes on LD 12, 18, and 22. The number of stillborn pups in the high-dose group was slightly non-statistically significantly increased, but this was due to a single dam delivering 9 stillborn pups. As previously mentioned, two high-dose dams had whole litter losses by LD 5.

The following clinical observations were noted only in offspring from the high-dose group: 6 pups (from 4 litters) that were cold to the touch; 4 pups (from 2 litters) that weren't nursing or nesting; 2 pups (from 1 litter) that were dehydrated; one pup that was emaciated; 2 pups (from 1 litter) with umbilical hernias; and one dam that did not remove the placenta from 3 pups.

As noted above for dams, separate tables listing 'FOB' findings for offspring were not provided,

and results of these observations were not discussed in the report.

TABLE 4. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	Control	5	50	100
Number of litters	25	25	22	24
Total number born	388	381	338	365
Number born live	386	381	336	354
Number born dead	2	0	2	9
Sex Ratio Day 1 (% ♂)	51.3±10.4	47.7±13.5	53.8±13.3	48.2±14.7
# Deaths Day 1 (%)	2 (0.5)	2 (0.5)	1 (0.3)	5 (1.4)
# Deaths Days 2-5 (%)	3 (0.8)/3 litters (12)	4 (1.0)/3 litters (12)	9 (2.7)/8 litters (36)	46 (13.2) **/13 litters (54)
# Deaths Days 6-12 (%) [b]	1 (0.4)	1 (0.4)	0 (0.0)	7 (3.2) #/7 litters
# Deaths Days 12-22 (%) [b]	0 (0.0)	0 (0.0)	1 (0.6)	6 (3.5) #/4 litters
Mean litter size: [c]				
Day 1	15.4±1.8	15.2±1.9	15.2±1.1	14.6±3.1 [14.8±3.0] [f]
Day 5 [d]	15.2±1.8	15.0±1.7	14.8±1.1	13.7±2.5 [12.6±4.6*] [f]
Day 5 [e]	10.0±0.0	10.0±0.2	10.0±0.0	9.8±0.5 [9.0±2.8] [f]
Day 12	10.0±0.2	9.9±0.3	10.0±0.0	9.5±0.8 * [7.1±2.2**] [f]
Day 18	8.0±0.0 g	8.0±0.0 g	8.0±0.2 g	7.5±0.9 * [6.9±2.3**] [h]
Day 22	8.0±0.0	8.0±0.0	8.0±0.2	7.5±0.9 * [6.8±2.4**] g
Viability index	98.7	98.4	97.0	85.6 **
Lactation index (LD 5-12)	99.2	99.2	100.0	95.9 **
Lactation index (LD 12-22) [i]	100.0	100.0	99.4	95.4 **

Data taken from Table B11, pp. 71 and 73-74, MRID 45377901.

a Data are given as Mean ± Standard Deviation, as appropriate.

b Calculated by reviewer and analyzed statistically using a 2 x 2 Chi-Square test.

c Excludes litters with no surviving pups.

d Before standardization (culling).

e After standardization (culling).

f Data are from 22 litters; excludes litters with no surviving pups; numbers in brackets=24 litters, includes litters with no surviving pups

g Data are from 20/22 litters; excludes litters that were not selected for further evaluation.

h Data are from 21/23 litters; excludes litters that were not selected for further evaluation.

i Excludes data from litters that were not selected for further evaluation.

Significantly different from control: * $p<0.05$; ** $p<0.01$; or # $p<0.05$, analyzed by reviewer.

2. <u>Body weight</u>: Offspring body weights during lactation are summarized in Table 5. The only treatment-related effects seen were slight decreases in the mean absolute body weights of high-dose females on PND 5 and high-dose males on PND 5-12 (5-6% decrease).

Offspring postweaning body weights are summarized in Table 6. High-dose males had a transient decrease in mean absolute body weight at PND 23 and 30 although the body weight gains of this group were similar to controls throughout the postweaning interval. There were no treatment-related effects on the absolute body weights or body weight gains of female offspring as compared to controls.

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

Postnatal Day		Dose (mg/kg/day)			
		Control	5	50	100
Absolute body weights					
1		6.1±0.4	6.1±0.5	6.1±0.5	5.8±0.6 (95)
5 b		8.8±1.0	8.7±0.8	8.7±0.9	8.3±0.9 (94)
5 c	Males	9.2±0.8	9.0±1.0	9.0±1.0	8.7±1.0 (95) [d] **
	Females	8.7±0.8	8.7±0.8	8.6±1.0	8.3±0.9 (95) *
12	Males	21.1±3.2	21.0±3.2	21.9±2.8	19.8±3.5 (94) *
	Females	19.8±3.5	20.2±3.0	20.8±3.0	19.2±3.5 (97)
18	Males	35.9±3.8	36.6±3.8	36.6±3.3	34.8±4.5
	Females	34.6±3.9	34.7±4.0	35.4±3.6	33.7±3.8
22	Males	47.8±4.9	49.0±6.2	48.6±5.1	45.8±6.2
	Females	45.9±5.1	46.2±5.4	46.5±5.5	44.2±6.4
Body weight gain					
5-22	Males	38.6±4.5	40.0±5.8	39.6±4.5	37.1±5.5
	Females	37.2±4.6	37.6±5.2	37.9±5.0	35.9±5.8

Data taken from Tables B11, C3, C4, C5, and C6, pp. 75, 184, 186, 188, and 190, respectively, MRID 45377901.
a Data are given as Mean ± Standard Deviation. b Before standardization (culling).
c After standardization (culling). d Numbers in parentheses equal percent of control; calculated by reviewer.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

TABLE 6. Mean post-weaning pup body weights and body weight gain (g) [a]

PND	Dose (mg/kg/day)							
	Control	5	50	100	Control	5	50	100
	Males				Females			
23	51.8±6.0	52.6±6.7	52.8±5.6	48.8±7.5 (94) [b] *	49.8±6.2	49.8±5.9	50.1±6.0	47.3±7.5 (95)
30	89.2±9.5	89.5±10.1	89.3±9.0	84.0±10.9 (94) **	82.3±8.9	82.6±8.2	82.4±8.9	78.9±10.6 (96)
44	205±23.4	208.0±20.6	206.7±19.2	199.2±22.8	159.8±13.6	160.0±14.0	160.9±13.2	156.9±15.9
58	323.9±32.6	323.5±27.8	326.3±25.1	316.2±28.3	208.8±18.7	210.3±19.4	213.1±17.3	207.9±20.6
72	402.7±37.7	405.8±34.2	409.3±28.6	399.6±34.9	244.5±21.9	244.5±23.0	249.2±19.4	244.6±24.9
23-79	372.2±48.5	384.9±33.7	384.8±30.0	370.7±34.0	206.5±20.1	203.6±17.2	213.5±21.1	208.2±28.6

Data taken from Tables C3, C4, C5, and C6, pp. 184-185, 187, 188-189, and 191, respectively, MRID 45377901.
a Data are given as Mean ± Standard Deviation.
b Numbers in parentheses equal percent of control; calculated by reviewer.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

3. Developmental landmarks/sexual maturation: Sexual maturation data are given in Table 7. There were no treatment-related effects on vaginal opening or balanopreputial separation.

TABLE 7. Mean age of sexual maturation (days) [a]

Parameter	Dose (mg/kg/day)			
	Control	5	50	100
Number evaluated (M/F)	60/60	60/60	60/59	57/56
Preputial separation (males)	48.4±3.6	48.0±2.7	48.4±2.9	47.6±2.9
Vaginal opening (females)	33.3±1.5	33.7±1.2	33.3±1.7	33.3±1.9

Data taken from Table C11, p. 196, MRID 45377901.
a Data are given as Mean ± Standard Deviation, as appropriate.

4. Behavioral assessments:

a. Functional observational battery: A formal FOB was not conducted on the offspring. Reported clinical observations included one mid-dose female with a displaced pupil (attributed histologically to lens luxation). There were no other clinical findings consistent with "abnormal autonomic functions."

b. Motor activity: Mean total activity count and mean time spent in movement data are summarized in Tables 8 and 9, respectively. Although substantial changes in motor activity were seen for several groups and time points during lactation (for example, decreases up to 52% in PND 18 males, increases up to 129% in PND22 males), none were statistically significant when compared to controls. It is likely that the large coefficient of variation during the PND17-22 period (61-107%) contributed to the lack of statistically significant findings. We also note that the values for all groups from PND14-22 (treated and control) are considerably below historical control range. These results, combined with the change in procedure for the current study (previous data included a 90-minute testing period, analyzed in 5-minute intervals), and the lack of positive control data, raise questions about the ability of the current procedure to detect compound-related changes.

Given these limitations of the data, we note that increases in motor activity were seen in mid- and high-dose females on PND 14 (123-127%), decreases were seen in males at all doses (48-75% of control levels) and in low dose females (76% of controls) on PND 18. High dose females had increased activity PND 18 (124% of control levels), and high dose males and females had increased activity on PND 22 (119-129% of control levels). Although the magnitude of some of these changes approaches biological significance (most notably the large decrease in mid-dose males at PND 18 [to 48% of control levels]), the relationship of these differences to treatment is difficult to determine, given the lack of dose-response relationships and the data limitations described above.

On PND 60, motor activity levels were similar across all groups.

No habituation was seen on day 14. On day 18, some habituation was seen in both sexes; this was more pronounced on PND 22. We note however, that evaluation of individual data revealed little habituation for many individual animals at various time points; reflective of the low levels of movement on PND 14, many individual animals showed little or no activity during the entire testing period at that time point.

TABLE 8. Mean motor activity data (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)				
	Control	5	50	100	Historical Control#
Males					
PND 14	78.2±82.1 [105%][b]	93.3±87.4	73.4±55.7	86.0±120.3 (110)	363 (189-576)
PND 18	245.2±205.8 [84%]	184.5±230.9 (75)	117.0±170.2 (48)	163.9±155.4 (67)	not provided
PND 22	202.8±132.0 [65%]	206.4±172.3	168.8±128.0 (76)	261.5±198.7 (129)	370 (241-513)
PND 60	730.0±83.5 [11%]	708.6±137.4	706.4±147.1	747.5±98.2	660 (475-782)
Females					
PND 14	124.7±134.0 [107%]	124.4±111.6	152.8±146.4 (123)	158.3±153.5 (127)	385 (226-553)
PND 18	250.9±214.1 [85%]	190.6±159.2 (76)	288.4±209.5 (115)	311.4±202.1 (124)	496 (305-667)
PND 22	266.4±161.6 [61%]	289.6±208.4	279.4±205.6	316.6±191.1 (119)	not provided
PND 60	752.6±122.9 [16%]	733.7±114.9	786.8±109.7	718.6±139.7	655 (532-748)

Data taken from Table F1, pp. 363-370, MRID 45377901.

Historical control data are from 1994-1999, presented as mean (range), taken from pp. 680-692; all presented historical control data were conducted in 90-min sessions; table values were calculated by reviewer by summing individual blocks for the first 60 minutes (12 5-minute blocks); this may not yield actual values for the first 60 minutes of any one study, since data were presented as mean, max, and min for each block.

a Data are given as Mean ± Standard Deviation, values in parenthesis indicate percent of control.

[b]Numbers in brackets represent coefficient of variation.

N = 20/20, 20/20, 20/20, 19/19 males/females for the control, low-, mid-, and high-dose groups, respectively.

TABLE 9. Mean motor activity data (time spent in movement [sec]) [a]

Test Day	Dose (mg/kg/day)				
	Control	5	50	100	Historical Control#
Males					
PND 14	43.4±58.3 [134%][b]	61.3±79.4	37.0±46.5 (85)	59.9±114.5 (138)	455 (151-771)
PND 18	280.6±299.8 [107%]	217.2±329.9 (77)	100.8±175.8 (36)	150.5±167.0 (54)	not provided
PND 22	210.8±158.4 [75%]	240.4±254.9 (114)	174.8±161.0 (83)	285.7±253.8 (136)	563 (328-819)
PND 60	1467.7±280.6 [19%]	1468.5±397.5	1469.9±404.4	1550.2±222.6 (106)	1453 (947-1751)
Females					
PND 14	87.3±127.4 [146%]	84.2±118.3	113.6±157.3 (130)	114.9±130.0 (132)	503 (311-667)
PND 18	242.6±248.2 [102%]	192.6±199.4 (79)	293.0±256.0 (121)	328.2±240.8 (135)	881 (538-1138)
PND 22	258.0±173.5 [67%]	304.6±227.3 (118)	284.8±231.2 (110)	342.8±247.6 (133)	543 (264-832)
PND 60	1539.4±369.0 [24%]	1499.8±261.1	1650.2±337.1	1564.9±466.5	1399 (1004-1655)

Data taken from Table F1, pp. 363-370, MRID 45377901.
Historical control data are from 1994-1999, presented as mean (range), taken from pp. 680-692; all presented historical control data were conducted in 90-min sessions; table values were calculated by reviewer by summing individual blocks for the first 60 minutes (12 5-minute blocks); this may not yield actual values for the first 60 minutes of any one study, since data were presented as mean, max, and min for each block.
a Data are given as Mean ± Standard Deviation, values in parenthesis indicate percent of control
[b]Numbers in brackets represent coefficient of variation.
N = 20/20, 20/20, 20/20, 19/19 males/females for the control, low-, mid-, and high-dose groups, respectively.

c. **Auditory startle reflex habituation:** The response magnitude and habituation data are given in Table 10. There were no statistically significant treatment-related differences between the treated and control groups on either testing day. However, for PND23 males and females, startle amplitude for block 1 was lower than controls in all treatment groups (61-90% of controls). Mean startle amplitude on PND23 was also somewhat decreased in all treatment groups, with the largest decrease for high dose females (71% of control mean). Habituation was seen over successive trial blocks in all groups on both days and was more pronounced on PND 61 than on PND 23. The latency or time to peak response was not measured or compared among groups.

TABLE 10. Auditory startle reflex response magnitude data (g) [a]

Test Day	Block	Dose (mg/kg/day)			
		Control	5	50	100
Males					
PND 23	1	18.9±10.0	13.4±7.2 (71)	14.3±6.9 (76)	17.0±9.5 (90)
	2	15.6±12.5	9.0±6.4	10.5±6.2	12.0±8.6
	3	12.9±10.3	7.9±6.7	9.3±5.6	11.3±8.0
	4	11.7±9.1	8.8±6.4	10.5±6.9	12.2±10.9
	5	13.2±9.9	8.8±8.6	10.9±8.3	12.3±9.9
	Average	14.5±9.6	9.6±6.4 (66)	11.1±5.8 (77)	13.0±8.0 (90)
PND 61	1	82.2±63.9	89.8±78.7	96.6±47.4	87.7±51.2
	2	52.3±51.2	59.6±48.3	63.6±67.6	64.4±57.0
	3	41.1±29.2	36.3±31.3	51.4±45.7	54.5±39.0
	4	32.9±19.7	32.4±25.2	50.0±40.6	43.9±30.7
	5	37.0±25.8	30.5±30.2	48.7±36.8	39.1±28.4
	Average	49.1±33.7	49.7±36.9	62.1±42.7 (126)	57.9±36.8 (118)
Females					
PND 23	1	19.7±16.4	15.3±9.3 (78)	15.4±7.7 (78)	12.1±6.2 (61)
	2	12.2±9.3	11.3±10.4	12.4±6.6	7.9±4.2 (65)
	3	10.2±7.8	10.3±11.6	9.6±5.6	7.2±4.9
	4	9.9±8.9	10.9±10.4	11.0±6.8	8.2±5.1
	5	11.2±8.1	9.9±9.9	9.3±7.0	9.4±8.2
	Average	12.6±8.6	11.6±9.4 (92)	11.6±5.6 (92)	9.0±4.6 (71)
PND 61	1	40.7±31.4	51.3±38.5	39.3±31.6	35.2±16.4 (86)
	2	27.6±29.0	35.7±35.7	30.1±24.1	31.4±43.4
	3	16.7±13.6	20.5±20.8	20.2±16.4	25.1±51.2
	4	13.1±10.8	15.7±12.2	16.8±13.4	20.6±27.8
	5	11.3±12.5	13.9±17.5	16.9±15.1	11.1±10.9
	Average	21.9±13.9	27.4±21.0	24.7±16.5	24.7±26.6

Data taken from Table F2, pp. 371-372, MRID 45377901

a Response magnitude = peak response - baseline response. Data given as Mean ± Standard Deviation, number in parenthesis represents percent of control.

N = 20/20, 20/20, 20/20, and 19/19 males/females for the control, low-, mid-, and high-dose groups, respectively.

d. Learning and memory testing:

1) **Passive avoidance:** The acquisition and retention data from the passive avoidance testing are given in Table 11. There were slight decreases in mean latency for session 1, trial 2 (79% of control for mid and high dose males, 81% of control for high dose females), and more pronounced decreases in latency for session 2, trial one, at all doses for both sexes (75/54, 52/50, and 68/85% of control for low, mid, and high dose [M/F], respectively). (although these differences are not statistically significant, the coefficients of variation are again high, making statistical significance difficult to achieve). Although the decreases are not strictly dose-related, they are consistent across both sexes. For comparison, the proportion of animals crossing within the first 20 seconds for Session 2, Trial 1(indicating failure to remember) was 30%, 40%, 75%, and 59% for males, 50%, 65%, 75%, and 67% for females, in control, low, mid, and high dose

groups, respectively. Similarly, the proportion of animals not crossing for 60 sec in the same trial (the maximum retention time measured) was 55%, 25%, 20%, and 32% for males, 30%, 5%, 10%, and 17% for females at the mid, low, and high doses, respectively. These deficits are considered treatment-related at all doses.

TABLE 11. Passive avoidance performance on PND 24-25 and 31-32 (mean ± S.D.) [a]

Session/Parameter		Dose (mg/kg/day)			
		Control	5	50	100
Males					
Session 1	Trials to criterion	4.2±0.7	4.2±0.9	4.6±2.0	4.4±0.9
	Latency trial 1 (sec)	4.8±3.3	5.0±4.9	5.4±3.4	4.8±3.3
	Latency trial 2 (sec)	27.0±20.5	24.2±21.0 (90)	21.2±19.3 (79)	21.4±20.2 (79)
	Failed to learn	0	0	0	0
Session 2	Trials to criterion	2.6±0.8	2.9±0.6	3.0±0.6	2.8±0.6
	Latency trial 1 (sec)	40.4±24.5	30.4±24.7 (75)	21.0±20.8 (52)	27.4±24.3 (68)
	No. crossing in 1st 20 sec	6	8	15	11
	No. not crossing in 60 sec	11	5	4	6
Females					
Session 1	Trials to criterion	4.2±1.0	4.2±0.8	4.2±0.8	4.3±0.7
	Latency trial 1 (sec)	4.4±2.6	5.0±3.7	4.3±2.2	4.8±2.6
	Latency trial 2 (sec)	23.8±17.0	28.2±15.2	23.2±18.3	19.3±13.8 (81)
	Failed to learn	0	0	0	0
Session 2	Trials to criterion	2.8±0.6	3.1±0.6	3.1±0.6	3.0±0.4
	Latency trial 1 (sec)	29.5±23.4	16.0±14.7 (54)	14.8±17.5 (50)	25.0±20.2 (85)
	No. crossing in 1st 20 sec	10	15	15	12
	No. not crossing in 60 sec	6	1	2	3

Data taken from Table E1, p. 335, MRID 45377901.
a Data are given as Mean ± Standard Deviation, as appropriate, number in parenthesis is percent control.
N = 20/20, 20/20, 20/20, 19/18 males/females for the control, low-, mid-, and high-dose groups, respectively.

2) **Water maze**: The acquisition and retention data from the water maze testing are given in Table 12. For low and mid-dose animals, there were no treatment-related effects on the number of trials to criterion, number of errors per trial, trial latencies, or the number of animals that failed to learn. For high dose animals, session 2, Trial 1 latency was increased in both sexes. Latency was 128% of controls in high dose males, 158% of controls in high dose females; in high dose females only, the number of errors per trial was also increased, to 171% of control levels (we note also the high coefficient of variation, leading to lack of statistical significan for these findings). This effect (decreased retention) is considered treatment-related, for high dose females.

TABLE 12. Water maze performance on PND 59-61 and 66-68 [a]

Session/Parameter		Dose (mg/kg/day)			
		Control	5	50	100
Males					
Session 1 [b]	Trials to criterion	8.4±2.5	9.0±3.4	8.7±1.8	8.8±2.9
	Errors per trial	0.40±0.24	0.46±0.24	0.39±0.20	0.47±0.34
	Latency trial 2 (sec)	20.2±14.0	19.8±15.3	17.2±10.8	20.9±15.9
	Failed to learn [c]	0	1	0	0
Session 2 [d]	Trials to criterion	6.2±2.4	6.6±2.6	5.8±1.1	5.8±1.5
	Errors per trial	0.10±0.17	0.15±0.22	0.09±0.13	0.07±0.10
	Latency trial 1 (sec)	10.4±10.6	12.8±7.2	10.0±5.6	13.3±8.8 (128)
Females					
Session 1 [e]	Trials to criterion	8.8±2.8	8.2±2.2	8.5±2.7	9.0±2.6
	Errors per trial	0.41±0.15	0.34±0.09	0.40±0.19	0.38±0.20
	Latency trial 2 (sec)	18.2±11.3	14.8±7.7	15.5±8.9	13.9±5.9
	Failed to learn [c]	2	0	1	0
Session 2 [f]	Trials to criterion	6.7±2.3	6.0±1.3	7.0±2.4	7.1±2.3
	Errors per trial	0.14±0.18	0.17±0.21	0.14±0.18	0.24±0.23 (171)
	Latency trial 1 (sec)	13.0±9.4	14.2±9.8	13.3±9.3	20.6±14.1 (158)

Data taken from Table E2, p. 336, MRID 45377901.

a Data expressed as Mean ± Standard Deviation, as appropriate, numbers in parenthesis represent percent of control values.
b N = 20, 20, 20, and 19, for the control, low-, mid-, and high-dose groups, respectively.
c Values for rats who failed to learn during session 1 were not included in means for session 2.
d N = 20, 19, 20, and 17, for the control, low-, mid-, and high-dose groups, respectively.
e N = 20, 20, 20, and 18, for the control, low-, mid-, and high-dose groups, respectively.
f N = 18, 20, 19, and 18, for the control, low-, mid-, and high-dose groups, respectively.

5. Postmortem results:

a. Gross necropsy: No treatment-related gross lesions were observed in the offspring at any scheduled sacrifice. 'No milk in stomach' was observed in pups from 0, 1, 3, and 5 litters, in control, low, mid, and high dose groups, respectively.

b. Brain and body weights: Mean brain and body weight data are presented in Table 13. Both at PND 12 and at PND 80-83, there were no statistically significant differences between treated and control groups for mean terminal body weights, brain weights, and brain-to-body weight ratios. For males only, there was a (non-significant) decrease in brain weight at the high dose (7%) on PND 12. A similar decrease was not seen in PND12 females. Examination of the individual data for PND12 males revealed that the lower mean value was largely attributable to 3 low outliers (0.765-0.959 g); pups with very low brain weight also had low body weight.

TABLE 13. Mean brain weight data [a]

Parameter	Dose (mg/kg/day)			
	Control	5	50	100
Males				
Postnatal Day 12				
Terminal body weight (g) [b]	21.6±3.6	20.9±3.3	22.0±2.4	19.4±4.2
Brain weight (g) [c]	1.235±0.107	1.224±0.097	1.278±0.095	1.154±0.162
Brain-to-body weight ratio (%) [c]	5.824±0.642	5.948±0.623	5.825±0.465	6.086±0.764
Postnatal Day 80+ [d]				
Terminal body weight (g)	451.6±50.5	464.8±28.5	435.4±28.4	437.3±38.8
Brain weight (g)	2.228±0.148	2.284±0.067	2.300±0.126	2.240±0.166
Brain-to-body weight ratio (%)	0.496±0.046	0.492±0.016	0.529±0.038	0.512±0.030
Females				
Postnatal Day 12				
Terminal body weight (g) [d]	18.8±3.9	20.2±3.1	20.8±3.2	19.0±4.5
Brain weight (g) [e]	1.139±0.127	1.202±0.112	1.210±0.108	1.162±0.125
Brain-to-body weight ratio (%) [e]	6.220±0.770	5.964±0.514	5.931±0.802	6.651±2.867
Postnatal Day 80+ [d]				
Terminal body weight (g)	259.2±25.7	259.6±22.8	268.5±25.0	261.4±28.4
Brain weight (g)	2.023±0.061	2.108±0.074	2.071±0.088	2.082±0.120
Brain-to-body weight ratio (%)	0.785±0.059	0.817±0.066	0.775±0.062	0.803±0.078

Data taken from Tables D3, D4, G3, and G4, pp. 317, 318, 465, and 466, respectively, MRID 45377901.

a Data given as Mean ± Standard Deviation.

b N = 20, 20, 20, and 19 animals for the control, low-, mid-, and high-dose groups, respectively.

c N = 20, 19, 19, and 19 animals for the control, low-, mid-, and high-dose groups, respectively.

d N = 20 for all groups.

e N = 20, 19, 19, and 19 animals for the control, low-, mid-, and high-dose groups, respectively.

c. Neuropathology

1) Macroscopic examination: There were no descriptions of gross lesions provided. The linear brain measurements of the control and high-dose animals are given in Table 14. At PND 80-83, the anterior/posterior measurement of the cerebrum was slightly but statistically significantly increased in mid- and high-dose male adult offspring. Although study authors did not consider this difference an adverse effect of treatment (according to the report, a dose-response pattern was not evident and, according to the author of the neuropathology report, the 0.4 mm difference between the group means is within the amount of error commonly seen on re-measurement of gross brain dimensions with a Vernier caliper), evaluation of the individual data clearly indicates a dose-related increase in this measurement. For example, 1, 3, 7, and 9/10 males in the control, low, mid, and high dose groups, respectively, had measurements ≥16.0 mm; 2, 8, 8, and 9/10, respectively, had measurements >15.5 mm. Similar, although less prominent, effects were seen in day 70+ females (measures were only provided for control and high dose): measures ≥ 16.0 were seen in 1 control and 3 high dose females, measures >15.5 were seen in 2 controls and 6 high dose females. Based on this analysis, the increase in ant/post cerebrum measurements are considered treatment-related.

TABLE 14. Mean gross morphometric data in offspring [a]

Parameter	Dose (mg/kg/day)					
	Control	5	50	100	Control	100
	Males				Females	
Day 12						
Ant/Post Cerebrum (mm)	12.3±0.57	n/a [b]	n/a	12.4±0.49	12.5±0.54	12.6±0.58
Ant/Post Cerebellum (mm)	5.6±0.36	n/a	n/a	5.4±0.43	5.7±0.50	5.6±0.42
Brain weight (g)	1.219±0.129	n/a	n/a	1.161±0.119	1.155±0.128	1.184±0.123
Day 70+						
Ant/Post Cerebrum (mm)	15.6±0.25	15.8±0.42	16.0±0.37 (103) [c] **	16.0±0.49 (103) *	15.6±0.34	15.7±0.33
Ant/Post Cerebellum (mm)	7.5±0.51	n/a	n/a	7.6±0.33	7.7±0.32	7.5±0.31

Data taken from Appendix A, pp. 511, 514-515, 559-560, MRID 45377901.
a Data are given as Mean ± Standard Deviation.
b Not available.
c Numbers in parentheses equal percent of control; calculated by reviewer.
N = 10 for all groups.
Statistically different from control: * $p<0.05$; ** $p<0.01$.

2) <u>Microscopic examination</u>: The qualitative histopathological findings are summarized in Table 15. In PND 12 animals of both sexes vesiculation, subventricular vesiculation, para-ventricular vesiculation, and para-aqueductal vesiculation were noted in various locations and represented foci of apoptosis, which are considered normal within the developing brain. The statistically significant decreased incidence of mild para-ventricular vesiculation of the thalamus in PND 12 high-dose males was attributed to a slight difference in the plane of the sections. There also appeared to be a slight increase in incidence of mild para-adqueductal vesiculation in the midbrain in treated males. None of the microscopic lesions were considered treatment-related, and all spinal cord and peripheral nervous tissue samples taken from F_1 adult offspring were found to be microscopically normal.

TABLE 15. Histopathology Findings

Parameter	Dose (mg/kg/day)	
	Control	100
Males		
Postnatal Day 12		
Thalamus - Mild para-ventricular vesiculation	10/10	5/10 *
Midbrain - Para-acqueductal vesiculation	2/10	4/10
minimal	1	1
mild	1	3
Postnatal Day 80-83		
Hydrocephalus	1/10	1/10
Females		
Postnatal Day 12		
Thalamus - Mild para-ventricular vesiculation	8/10	9/10
Midbrain - Para-acqueductal vesiculation	1/10	2/10
minimal	1	1
mild	0	1
Postnatal Day 80-83		
Vestibular nucleus/pons - mild inflammation and gliosis	1/10	0/10
Mild intraventricular hemorrhage	0/10	1/10
Hydrocephalus	2/10	0/10

Data taken from Appendix A, pp. 516-526, 561-570, MRID 45377901.
Statistically different from control: * $p<0.05$; ** $p<0.01$.

Mean microscopic morphometry data are given in Table 16. The mean measurements taken from the high-dose male and female pups killed on PND 12 and high-dose males killed on PND 80-83 were similar to those of their respective control groups. The mean cerebellar height of high-dose females killed on PND 80-83 was slightly but statistically significantly greater than that of controls, and the difference persisted even when the measurement method was adjusted to account for artifactual separation of cerebellar folia due to tissue shrinkage during processing. The author of the neuropathology report stated that this difference was unlikely to represent an adverse effect of treatment for the following reasons: any interruption in normal development of the cerebellum would be more likely to cause a decrease in cerebellar height rather than an increase; a treatment-related effect on the anterior-posterior measurement of the cerebellum would be more predictive of an alteration in the development of this brain region, and such a change was not noted here; and a similar change was not noted in males. We note, however, that examination of the individual data revealed that 7/10 measurements for high dose females were outside (above) the range of control values. Morphometric measurements for cerebellar height of intermediate dose groups should be provided.

TABLE 16. Mean microscopic morphometric data in offspring [a]

Parameter	Dose (mg/kg/day)			
	Control	100	Control	100
	Males		Females	
Day 12				
Frontal Cortex (µm)	1265±56.63	1282±104.4	1272±113.7	1229±89.66
Dentate Gyrus (µm)	984±57.7	979±102.3	960±59.9	946±61.1
Cerebellar Height (µm)	3048±345.6	2976±346.9	3040±137.9	3014±307.6
Day 70+				
Frontal Cortex (µm)	1800±127.0	1762±88.5	1711±60.98	1699±82.21
Dentate Gyrus (µm)	1584±104.9	1632±37.5	1495±39.27	1505±78.42
Cerebellar Height (µm)	5309±402.36@	5213±301.2	4949±61.76	5165±205.2 (104) [b] **

Data taken from Appendix A, pp. 514-515, 559-560, MRID 45377901.
a Data are given as Mean ± Standard Deviation.
b Number in parentheses equals percent of control; calculated by reviewer.
N = 10 for all groups (except n=9 for day 12 females, cerebellar height).
@ Standard deviation provided in the summary table was incorrect; value was recalculated by reviewer.
Statistically different from control: * $p<0.05$; ** $p<0.01$.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that the maternal NOAEL was 5 mg/kg bw/day, based on decreased body weight, body weight gains, and food consumption, and the offspring NOAEL was 50 mg/kg bw/day, based on decreased pup survival and body weight. The study author also concluded that under the conditions of this study there was no evidence of developmental neurotoxicity.

B. REVIEWER COMMENTS:

Maternal toxicity was seen only at the high dose, and was limited to small decreases in body weight (4-6%), body weight gain (83% of controls during GD6-21), and food consumption (87% of control levels during gestation, 93% during lactation days 1-14). The only other possibly treatment-related finding was 'hyperreactivity' seen in two high dose females on the first day of dosing. Although investigators stated that the maternal NOAEL was 5 mg/kg/day, based on decreased body weight gain, we find that the decrease in body weight at that dose (a difference of 3.4 g in absolute weight, not statistically significant) was of insufficient magnitude to be considered toxicologically significant.

There was a decrease in offspring viability during lactation, with a significant increase in pup death during days 2-5 (46 deaths/13 litters), 6-12 (7 deaths/7 litters), and 12-22 (6 deaths/4

litters) at the high dose (in controls there were 3 deaths/3 litters on days 2-5, and 1 death on days 6-12). There was also a slight increase in deaths during days 2-5 (9 deaths/8 litters) at the mid dose (not statistically significant). Clinical signs seen in pups at the high dose (cold to touch, not nursing, emaciation) are consistent with the increase in pup death at that dose. Slight differences in body weight (5-6% decrease) were also seen in high dose pups during early lactation (day 5, males and females; day 12, males only) and up to day 30 (males only).

Although there were no statistically significant changes in motor activity, there were substantial differences between control and treated groups at several time points (for example, decreases in males of all treatment groups on PND 18 [48-75% of control levels], increases in mid-dose females [115-123% of controls on PNDs 14 and 18], and increases high dose animals of both sexes [119-129% of control levels] on PND 22 for males, PNDs 14, 18, and 22 for females). Problems with the data (including very low levels of activity compared with historical controls and high levels of variability at all time points) make interpretation of these results difficult, as discussed previously.

Slight decreases in auditory startle were also seen in all treated groups on PND23; these findings were not dose-related in males, and only slightly dose-related in females (for the first block, amplitudes were 78% of control at low and mid-dose, 61% of control at high dose). These decreases are considered treatment-related for high dose females only, on PND23.

Decreases in performance of the passive avoidance task were seen at all doses, for both sexes, for PND 24-25/31-32 pups. Although high variability again precluded a finding of statistical significance, examination of individual data, including distribution of latency values, supported the deficits in performance at all doses, which were greater for the retention phase than for initial learning. Performance of the water maze task at PND 59-61/66-68 was not impaired, except for a remaining deficit in retention in the high dose females.

Neuropathologic evaluations revealed a dose-related increase in anterior/posterior cerebrum measurements for mid- and high-dose adult males, and an increase in cerebellar height for high dose adult females (low and mid-dose were not evaluated).

In summary, impairments in learning, immediately post-weaning, were seen in pups at all doses. Additional effects seen at higher doses include decreased pup survival and increases in morphometric measurements. Changes in motor activity could not be evaluated, due to problems with the data.

C. STUDY DEFICIENCIES:

Major deficiencies:

1) The method used for detection of functional changes 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), when testing was done with respect to time of test substance administration, what the environmental conditions were (e.g., noise level, etc.), whether scoring criteria were used for the measured parameters, the duration of the observation period for open

field observations. There was no mention of evaluation of pupillary function such as constriction of the pupil in response to light, or a measure of pupil size.
2) The motor activity procedure used in the current study varied from that previously used by this laboratory; no positive or historical control data for the current procedure were provided. In addition, control activity levels in the current study were considerably below the range of provided historical control data, even after adjustment for the change in testing interval. No discussion of this discrepancy was provided.
3) Acceptable positive control data were not provided.
4) Morphometric measurements of female cerebellar height for day 12 should be provided for intermediate dose groups, as discussed above.

Minor deficiencies:
1) We noted an excursion outside the specified temperature range (to 100°F), which appears to have occurred on one of the test days of water maze testing. No explanation or comment on this excursion was provided in the report; comment should be provided regarding possible effects of this temperature excursion on the results of the water maze testing.
2) We note that statistical analyses for homogeneous data were performed using ANOVA at $p<0.001$. Explanation should be provided as to why the analyses were not performed at $p<0.05$.
3) We note that according to the histopathology report, gross morphometric measurements (A/P cerebrum and cerebellum) were performed on all brains evaluated histopathologically, however data were reported only for high dose and control animals, except for A/P cerebrum in mid and low dose males. We also note that morphometric measurements were made bilaterally for hippocampus, but only the mean values were reported. Values for morphometric measurements evaluated, but not included in the current report, should be submitted.
4) Latency was not reported for auditory startle data.
5) Compound stability for cymoxanil technical was not reported.

The maternal LOAEL is 100 mg/kg/day, based on slight decreases in body weight (4-6%), body weight gain, and food consumption. The maternal NOAEL is 50 mg/kg/day.

The offspring LOAEL is 5 mg/kg/day, based on decreased retention in the passive avoidance task. The offspring NOAEL is not determined.

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). Classification may be upgradable to guideline upon submission of additional information to address deficiencies listed above, including: 1) procedural information for functional observation procedures; 2) appropriate positive control data; 3) additional morphometric measurements, as described; 4) additional information regarding motor activity data, as described; 5) additional information regarding temperature excursion; 6) explanation of statistical procedures, as described.

Appendix – Positive Control Data

The following positive control data were provided:

Parker, R. (1999) Neurotoxicity evaluation of positive control substances in Crl:CD®(SD)IGS BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-075, August 6, 1999. Unpublished.
This study used a functional observational battery (FOB) to evaluate the positive control substances acrylamide, trimethyltin, MK-801, Carbaryl, and DDT and was not acceptable for use with the current study because the current study did not use a formal FOB.

Foss, J. (1992) Neurotoxicity evaluation of DDT in Crl:CD®(SD)IGS BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-015. Unpublished. This study used an FOB to evaluate the positive control substance DDT and was not acceptable for use with the current study because the current study did not use a formal FOB.

Lochry, E., J. Foss, and M. Christian. (1990) Validation of a functional observational battery and motor activity measure using positive control substances. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 11th annual meeting of the American College of Toxicology; Orlando, Florida; October, 1990. This study used an FOB to evaluate the positive control substances DDT, physostigmine monosalicylate, or acrylamide and used a motor activity assessment to evaluate the positive control substances chlorpromazine or amphetamine. It was not acceptable for use with the current study because the current study did not use a formal FOB, and because the motor activity sessions in the positive control study were 2 hours in duration and comprised of 24 5-minute blocks, while the current study used 1 hour sessions comprised of 6 10-minute blocks. There was also insufficient information provided to determine whether the same equipment was used as was used in the current study.

Foss, J. (1991) Neurotoxicity evaluation of positive control substances in Crl:CD® VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-014. Unpublished. This study used an FOB and motor activity assessment to evaluate the positive control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon. It was not acceptable for use with the current study because the current study did not use a formal FOB, and because the motor activity sessions in the positive control study were 1.5 hours in duration/comprised of 18 5-minute blocks, while the current study used 1 hour sessions comprised of 6 10-minute blocks. There was also insufficient information provided to determine whether the same equipment was used as was used in the current study.

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.

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.

Lochry, E. and E. Riley (1980) Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. Neurobehavioral Toxicology, Vol. 2, pp. 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., 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.

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.

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.

E. Lochry, A. Hoberman, and M. Christian (1985) Detection of prenatal effects on learning as a

function of differential criteria. 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.

Section 21: Neuropathology Validation included a brief description of the consulting neuropathologist's credentials, experience, and publications, and also included the neuropathology methods and results from Argus Research Laboratories, laboratory project number 012-058 [mentioned above].

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
		dev neurotox	rats		oral	diet						Maternal
		dev neurotox	rats		oral	diet						Offspring