7/25/1998

DATA EVALUATION RECORD

012987

N-Methylneodecanamide

Study Type: §83-3; 83-6; Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential)/Neurotoxicity Study - Rat

Work Assignment No. 2-47K (MRID 43883914)

Prepared for

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

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Sandra Daussin

Signature: Sandra Daussin
Date: 7/18/97

Secondary Reviewer:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 7/21/97

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 7/22/97

Quality Assurance:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 7/22/97

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10/3/97

EPA Reviewer: Laurence D. Chitlik, DABT
Toxicology Branch I (7509C)

EPA Secondary Reviewer: Marion Copley, DVM
Registration Branch I (7509C)

7/25/96

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential)/Neurotoxicity Study - Rat
OPPTS Number: 870.3800 OPP Guideline Numbers: §83-3, §83-6

DP BARCODE: D228410 SUBMISSION CODE: S507937
P.C. CODE: 079052 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): N-Methylneodecanamide (95.9% a.i.) (Purity of the same lot, number 21709-17, of the test material was given as 96.04% in MRID 43883913)

SYNONYMS: None

CITATION: Hoberman A.H. (1993) Developmental Toxicity (Embryo-Fetal toxicity and teratogenic potential) including a Developmental Neurotoxicity Evaluation of Sample No. 38674 administered orally via gavage to Crl:CD BR VAF/Plus presumed pregnant rats GLP Study 91-003. Argus Research Laboratories Inc., Horsham PA. Laboratory Study number 91-03, October 19, 1993. MRID 43883914. Unpublished

SPONSOR: Colgate-Palmolive Company, Piscataway NJ

EXECUTIVE SUMMARY: In a combined developmental toxicity (embryofetal toxicity and teratogenic potential) and developmental neurotoxicity study (MRID 43883914), N-methylneodecanamide (lot number 21709-17, 95.9% a.i.) was administered via gavage to 50 female Crl:CD BR VAF/Plus (Sprague-Dawley®) rats/dose at levels of 0, 40, 125, or 400 mg/kg/day on days 6 through 15 of presumed gestation for developmental toxicity evaluations (25 rats/sex/dose) or on days 6 of presumed gestation through day 11 of lactation (postpartum) for developmental neurotoxicity evaluations (25 rats/sex/dose). As requested by OPPTS, this DER addresses both the developmental toxicity and developmental neurotoxicity components of this study (§83-3, §83-6).

Dams in the 400 mg/kg/day dose group exhibited treatment-related mortality, increases ($p \leq 0.01$) in the incidence of clinical signs of toxicity (excess salivation, rales, ataxia, urine stained fur

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and decreased motor activity), reductions in the mean body weights on gestation days 9-16 (3.1-5.0%, $p \leq 0.01$ or 0.05), a 20.9% decrease ($p \leq 0.01$) in body weight gain for gestational days 6-16 (body weight gain increased over days 0-22 of lactation), decreases in feed consumption during gestation days 6-20 (13.7%, $p \leq 0.01$) and 0-20 (8.2-8,8%, $p \leq 0.05$) and days 8-12 of lactation (8.2%, $p \leq 0.01$).

At the 125 mg/kg/day dose level, maternal toxicity was characterized by treatment-related clinical signs (excessive salivation and rales) and slight (1.3%), but statistically significant ($p \leq 0.01$) reductions in feed consumption during the common treatment interval (days 6-9 of gestation).

Developmental toxicity was noted by a 4.6% statistically significant reduction in mean body weights in caesarean-derived male fetuses from dams administered 400 mg/kg/day test article from days 6-15 of gestation, a 7.3-11.7% statistically significant reduction in mean body weights in the naturally delivered F_1 generation pups on days 5-22 of lactation and a 13.2% increase in brain/body weights on day 12 postpartum from dams administered 400 mg/kg/day test article from day 6 of gestation through day 11 of lactation (postpartum). The reduced body weights were greater in males than females. Although the lactational body weights were combined, the post-weaning body weights were especially depressed in the high dose males. These pup body weight effects persisted long after the treatment was discontinued in the dams, although feed consumption in all dose groups was comparable to the controls during post-weaning.

Neurotoxicity was suggested by minimally, but statistically significant affected motor activity on day 18 postpartum in male pups from dams administered 400 mg/kg/day test article from day 6 of gestation through day 11 of lactation (3/18 blocks for number of and 2/18 blocks for time spent in movement). In addition at day 18 postpartum, in all 18 blocks for males and in 14 of 18 blocks for females, time and number of movements were increased in the high-dose as compared to the controls, although the differences were statistically significant only at the blocks noted above for males. On day 14, the time and number of movements were increased for females at all dose levels compared to the controls; the differences were not statistically significant. Motor activity measurements at days 22 and 61 were comparable to the controls at all dose levels. The increases in motor activity at days 14 and 18 are considered transient indications of neurotoxicity.

The maternal LOAEL is 125 mg/kg/day, based on clinical signs of toxicity (including salivation, rales, ataxia, urine stained fur and decreased motor activity) and reduced mean feed consumption values. The maternal NOAEL is 40 mg/kg/day.

The developmental LOAEL is 400 mg/kg/day, based on reduced c-section-derived male fetuses, reduced pup weights (both sexes) during days 5-22 postpartum and in males on days 1-22, post-weaning. The developmental NOAEL is 125 mg/kg/day.

The developmental neurotoxicity LOAEL is 400 mg/kg/day, based on increases in motor activity measurements, only in male pups (only on day 18). Effects were transient and did not persist after dosing ceased

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after day 22 postpartum. The neurotoxicity NOAEL is 125 mg/kg/day.

This developmental toxicity/neurotoxicity study in the rat is classified **Acceptable (guideline)** and does satisfy the guideline requirement for a developmental toxicity/neurotoxicity study (OPPTS 870.3800, §83-3 (a) and §83-6) in the rat. However the registrant should 1) explain the discrepancy between the purity listed in this MRID (95.9%) as compared to that listed for this study in MRID 44211902 (95.8%) and the reproduction study with the same lot (96.08%) and 2) provide acceptable analytical data to confirm the purity of the test material and the actual concentrations of the dosing formulations.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: N-Methylneodecanamide (Sample Nos. 38763, 38764-37, 38764-38 and 38679-39)
   Description: Light viscous liquid, stable at room temperature in plastic bottles protected from light for an unspecified interval
   Lot #: CC #21709-17
   Purity: 95.9% a.i. (see deficiency section)
   CAS #: 105726-67-8
   Structure: Not provided.

2. Vehicle: Propylene glycol
   Lot #: Not provided by sponsor
   Purity: Not provided by sponsor

3. Test animals: Species/Sex: rat/female
   Strain: Crl:CD BR VAF/Plus (Sprague-Dawley®)
   Age at mating: Males 43-57 days, Females 92 days
   Weight at Day 0 presumed gestation: Males 426-1138 g, Females 205-319 g
   Source: Charles River Laboratories, Inc., Males-Portage MI, Females-Raleigh NC
   Housing: Individually (except during mating) in wire bottom stainless steel cages suspended above absorbent paper liners. Females with pups were housed in polycarbonate nesting boxes with Bed-O'-Cobs® bedding.
   Diet: PMI® Feeds Certified Rodent Diet (#5002), ad libitum
   Water: Tap water processed through a reverse osmosis membrane, ad libitum
   Environmental conditions:
      Temperature: 70-78°F (occasional short-term deviations of up to 61-82°F)
      Humidity: 40-70 % (occasional short-term deviations of up to 20-78% RH)
      Air changes: 10/hr (minimum), HEPA filtered
      Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P_1): 13 days

B. PROCEDURES AND STUDY DESIGN

Design Overview: The purpose of this study was to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) following oral administration (gavage) of n-methylneodecanamide to presumed pregnant rats and provide information on the potential neurotoxic effects in offspring following exposure in utero and via the mother's milk. Female (P_0) rats designated for caesarean-sectioning (c-sectioning) on gestation day 20 were administered the test article on gestation days 6-15. Female P_0 rats designated for natural delivery and subsequent weaning of F_1 pups were administered the test article from gestation day 6 through lactation day 11 (postpartum) and maintained until sacrifice on day 22 postpartum. Maternal rats were evaluated for mortality, clinical signs of toxicity, body weights, feed consumption values and selected reproductive parameters. Pups delivered by c-sectioning were evaluated for fetal weights, gender and soft tissue and skeletal alterations. Pups delivered naturally were evaluated for viability, clinical observations,

weights, feed consumption, external alterations, sexual maturation, a battery of neurotoxicity assays, sex organ and brain weights and histopathologic changes.

1. In life dates - start: November 18, 1991 end: March 13, 1992

2. Mating procedure: One male was caged with one female from the same test group. Females with spermatozoa observed in a smear of the vaginal contents or a copulatory plug in situ were considered to be at day 0 of presumed gestation.

3. Animal assignment: After cohabitation, a computer-generated (weight-ordered) randomization procedure was used to assign (by body weight) 50 mated rats to four test groups (Table 1). A second computer-generated randomization procedure was then used to designate 25 rats/group for c-sectioning on gestation day 20 and 25/group for natural delivery and rearing to day 22 postpartum.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	50
Low	40	50
Mid	125	50
High	400	50

4. Dose selection rationale:

The concentrations tested were selected by the sponsor based on data obtained in two range-finding studies (Argus Research Laboratories Studies #403-007P and 403-011P). In the first range-finding study, doses of 0, 5, 15, 50 or 100 mg/kg/day were orally administered (gavage) in propylene glycol to presumed pregnant rats on days 6-15 of presumed gestation. Rats were examined daily for viability, clinical observations, abortions, premature deliveries and body weights during the dosage and post-dosage periods. Feed and water consumption values were recorded daily on days 0-20 of gestation. At sacrifice, all rats were subjected to a gross necropsy of the thorax and abdomen. The number and distribution of implantations, live and dead fetuses, early and late resorptions and corpora lutea/ovary were recorded. No maternal or developmental toxicity was seen at any dose level. As there was no overt maternal or developmental toxicity at dose levels up to 100 mg/kg/day, a second range-finding study was conducted.

The second range-finding study was designed the same as the first range-finding study, except that doses of 0, 50, 100, 150, 300 and 450 mg/kg/day were tested. Maternal toxicity was observed at the 100-450 mg/kg/day dose levels (ataxia, rales, excess salivation) and maternal rats in the 450 mg/kg/day dose group also had reduced body weight gain and feed consumption values. No c-sectioning or litter

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parameters were affected at the highest dose tested. It was concluded that the appropriate dose levels for a full developmental toxicity study were as high as 450 mg/kg/day.

5. Dosage preparation and analysis

Solutions of the test substance were prepared by the sponsor in propylene glycol. The prepared test substance solutions and vehicle were delivered to the test laboratory on November 12, 1991 and stored at 4°C, protected from light until use.

Results - Homogeneity analysis: No homogeneity determinations were done for this study.

Stability analysis: At 4°C for 8 weeks, 0.100, 5.00 and 22.11 mg/ml solutions of the test material were 99.0, 96.4 and 99.8% of their target concentrations.

Concentration analysis: The measured concentrations of the test article in the 0.8, 2.5 and 80 mg/ml solutions were 98.8, 98.4 and 98.0% of their target concentrations, respectively.
Raw data for concentration analyses were not reported; only a summary table is presented. No methodology was indicated. Analytical data presenting the purity of the test substance and the actual concentration of the dosing formulations must be provided. The registrant should submit data to indicate the analytical methodology used, when samples were taken, where samples were analyzed, and by whom.

6. Dosage administration: All doses were administered once daily by gavage, on presumed gestation days 6 through 15 for the dams designated for c-sectioning and from presumed gestation day 6 through day 11 of lactation (postpartum), in a volume of 5 ml/kg body weight adjusted daily based on individual body weights recorded immediately before intubation.

C. OBSERVATIONS

1. Parental animals: All animals were observed daily during the dosage and postdosage periods for viability (at least twice daily), clinical signs of toxicity, abortions and premature delivery. During the dosage period rats were also examined for signs of autonomic disfunction, abnormal postures, abnormal movements or behavioral patterns and unusual appearance. Maternal body weights and feed consumption values were recorded daily during the dosage and postdosage periods and on day 0.

Estrous cycling (vaginal cytology) was evaluated for one week before cohabitation and up to 21 days during cohabitation until day 0 of presumed gestation. Mating performance was evaluated daily during cohabitation and confirmed by implantation sites present at sacrifice or natural delivery of a litter.

Maternal behavior of all dams that naturally delivered litters was recorded during daily dosing.

At necropsy for rats designated for c-sectioning (day 20 of gestation), the thoracic and abdominal viscera were examined for gross lesions and the uterus was removed and examined

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for the number and distribution of implantations, early and late resorptions, and live and dead fetuses. The number of corpora lutea in each ovary was recorded, each fetus was weighed and examined for sex and external alterations. Live fetuses were sacrificed and approximately 1/2 the fetuses in each litter were fixed in Bouin's solution and examined for soft-tissue alterations (a modification of Wilson's sectioning) and the remaining fetuses from each litter were cleared, stained with Alizarin Red S and examined for skeletal alterations.

Rats that delivered naturally were sacrificed on day 22 postpartum. Rats designated for natural delivery that did not deliver a litter were sacrificed on day 25 of presumed gestation and examined for gross lesions and implantation sites.

2. Fetal observations: The F_1 generation litters delivering naturally were observed twice daily for viability and daily for general appearance and body weights were recorded on postpartum days 1 (birth; postnatal day 0 or day 0 of lactation), 5 (pre- and postculling), 8, 12, 14, 18 and 22. Body weights and feed consumption values were recorded weekly after weaning. On the days the F_1 generation rats were weighed or handled, they were also examined for signs of autonomic disfunction, abnormal postures, abnormal movements or behavior patterns and unusual appearance to the extent that these evaluations could be made at each age.

On day 5 postpartum, the F_1 generation litters were culled to 4 pups/sex and on postpartum day 12, ten pups/sex/dose were selected for brain weight measurements. Of these, six pups/sex/dose were selected for neurohistopathologic evaluation of the olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum and thicknesses of cellular layers in the neocortex, hippocampus and cerebellum. Three pups/sex/dose were selected from each litter for continued observation. These selections were done using tables of random numbers and the pups were sacrificed on day 71 post-weaning. The 3 pups/sex/litter continued on study were evaluated for viability, survival, body weights, feed consumption and sexual maturation (testes descent, preputial separation or vaginal patency). One pup/sex/litter was evaluated behaviorally; motor activity (on postpartum days 14, 18, 22 and 61), auditory startle habituation (on approximate postpartum days 23 and 61) and tests of learning and memory beginning at 23 to 24 days of age (passive avoidance and watermaze testing).

All maternal rats and pups not selected for these measurements were sacrificed on day 22 postpartum and examined for gross lesions (including cross-sectioning of the skull and examination of the brain for hydrocephaly). The number of implantation sites observed in each dam was recorded.

Motor activity movements for each rat were monitored by a passive infrared sensor mounted outside a wire-bottomed stainless-steel cage for 1.5 hours/test period. A set of up to 32 cages and sensors was controlled by a microcomputer which sampled the output of each sensor and calculated the number and total duration of movements at five-minute intervals.

Auditory startle habituation was evaluated in groups of four in sound-attenuated chambers. Each rat was placed in a cage

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mounted above a platform containing a force transducer in the base. A microcomputer sampled the transducer output and controlled the test session. The rats were initially given an adaption period of five minutes followed by a series of 20 msec of 120 dB bursts of noise at 10 second intervals for 50 trials. Baseline trials were given to sample the baseline force in the absence of a stimulus during the last minute of the adaptation period (10 trials) and immediately following the stimulus trials (10 trials). The peak amplitude of each response was recorded and the average response on baseline trials was subtracted to calculate the response magnitude. The average response magnitude and the pattern of responses over 50 trials (10 blocks) were compared across dosage groups.

Passive-avoidance was evaluated using a two-compartment chamber with hinged plexiglass lids. One compartment contained a bright light and a plexiglass floor and, separated by a sliding door, the other compartment contained a grid floor to which a 1 second pulse of 1 mA electric current was delivered. During each trial, the rat was placed in the "bright" compartment, the door was opened and the light turned on. The rat explored the apparatus until it entered the "dark" compartment. The door was then closed, the light turned off and a pulse of current was delivered. The rat was then removed to a holding cage for 30 seconds before the start of the next trial. Trials were repeated until the rat remained in the "bright" compartment for 60 seconds on each of 2 consecutive learning trials. A maximum of 15 trials/rat/day were given. The number of trials required to reach the criterion and the latency in each trial were recorded on both days of testing.

Watermaze testing evaluated overt coordination, swimming ability, learning and retention. During each trial, the rat was placed in the starting position (base of the stem furthest from the two arms of a water-filled stainless-steel "M" maze) and was required to swim to the correct goal of the maze in order to be removed from the maze. Each rat was required to enter both arms of the of the maze on the first trial. The second arm entered on the first trial of the first day of testing was designated as the correct goal for all subsequent trials during both test sessions. All rats were tested on two days, separated by an interval of one week. The latency (seconds) required to reach the correct goal and the number of incorrect turns in the maze (errors) in each trial was recorded. Trials were repeated until each rat reached a criterion of 5 consecutive effortless trials. Each trial was separated by a 15-second interval, during which the rat was placed in a holding cage. Rats not entering the correct goal within 60 seconds were guided to this goal, removed from the water, and assigned the maximum latency value of 60 seconds. Rats failing to reach the criterion within 15 trials during either day of testing were assigned a maximum score of 15 trials.

F_1 generation rats selected for neurohistological evaluation were killed by administering a combination of heparin and a barbiturate, perfused <u>in situ</u> and dissected to expose the brain, spinal cord and peripheral nerves in the hindlimbs. Sections of the brain, Gasserian ganglion, cervical, thoracic and lumbar regions of the spinal cord (with dorsal roots from the thoracic and cervical segments) and sections of the sciatic, tibial, fibular and sural nerves were evaluated. Morphometric analyses were also conducted to measure the thickness of cellular layers of the neocortex, hippocampus and cerebellum. Neurohistopathologic

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examinations were conducted on these tissues from the control and high-dose group rats.

At 74-93 days of age, the F_1 rats selected for continued evaluation were sacrificed after these tests were conducted.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices:

Reproductive indices: The following indices were calculated from c-sectioning and natural delivery records of animals in the study:

C-Sectioning Indices:
- % rats pregnant
- % rats found dead
- % dams with any resorptions
- % dams with viable fetuses

Natural Delivery Indices:
- % rats pregnant
- % delivered litters
- % dams delivering on gestation day 21
- % dams delivering on gestation day 22
- % dams delivering on gestation day 23
- % dams with stillborn pups
- % dams with all pups dying days 1-4 postpartum
- % dams with all pups dying days 5-22 postpartum
- % dams with all pups dying before day 22 postpartum

Additional data collected for c-sectioning dams included numbers of rats assigned to c-sectioning, pregnant, pregnant and c-sectioned on day 20 of gestation, corpora lutea, implantations, litter sites, live and dead fetuses, early and late resorptions, dams with any resorptions and dams with all conceptuses resorbed.

Litter Indices:
viability
lactation
- % stillborn pups
- % pups of unknown vital status
- % pups dying on day 1
- % pups dying on days 2-5
- % pups dying on days 6-8
- % pups dying on days 9-12
- % pups dying on days 13-14
- % pups dying on days 15-18
- % pups dying on days 19-22
- % male live fetuses/litter
- % resorbed conceptuses/litter

Additional data collected for dams delivering naturally included numbers of delivered litters with one or more liveborn pups, total, liveborn, stillborn and pups of unknown vital status, pups dying on days 1, 2-5, 6-8; 9-12, 13-14, 15-18 and 19-22, surviving pups/litter on days 1 and 5 (preculling), litters postculling on days 5, 8, 12, 14, 18 and 22, live litter size at weighing and pup weight on days 1 and 5 (preculling) and postculling on days 5, 8, 12, 14,

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18 and 22.

Additional data collected for litters obtained from c-sectioning and delivered naturally included (numbers of litters with one or more fetuses, implantations, live fetuses, live male fetuses and live fetal body weights (males and females).

3. Historical control data: Acceptable historical control data for this study are found in Appendix M, p.p. 940-1099.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: Clinical signs of toxicity in the P_0 generation rats considered to be treatment-related are summarized in Table 2. One female in the 400 mg/kg/day dose group was found dead on day 9 of gestation. After the first or second dosages, this rat exhibited ataxia, excess salivation, weight loss and reduced feed consumption. No gross lesions were found in this female at necropsy and it had a litter of 17 embryos that appeared to be normal for their developmental ages.

   Rats in the 125 and 400 mg/kg/day dose groups exhibited dose-related incidences of excess salivation and rales. Ataxia, urine-stained abdominal fur and decreased motor activity were also observed in the high-dose group. The incidences of all these clinical signs of toxicity were statistically significant in the 400 mg/kg/day dose group rats. Single observations of excess salivation or rales seen in the 40 mg/kg/day dose group rats were not considered to be treatment-related because they were comparable to incidences seen in the control group rats and they were only observed once.

Table 2. Clinical signs of toxicity P_0 generation females assigned to caesarean-sectioning and natural delivery[a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (50)	40 (50)	125 (50)	400 (50)
Excess Salivation	0/0[b]	1/1	9/9	55/26**
Rales	1/1	1/1	2/2	11/9**
Ataxia	0/0	0/0	0/0	93/36**
Urine-Stained Fur	0/0	0/0	0/0	9/3**
Decreased Motor Activity	0/0	0/0	0/0	3/3**

a Data extracted from study report Table B1, page 75
b -/- = (days x rats)/rats observed per group
** Statistically significant $p \leq 0.01$

2. Body Weight: Maternal body weight gain data for rats assigned to both c-sectioning and natural delivery are summarized in Table 3. In the 400 mg/kg/day group females, statistically significant reductions in mean body weights were seen on gestation days 9 (5.0% decrease, $p \leq 0.01$), 12 (3.9% decrease, $p \leq 0.01$) and 16 (3.8% decrease, $p \leq 0.05$). In

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addition, a 20.9% decrease ($p \leq 0.01$) in body weight gain for gestational days 6-16 were also seen in this dose group rats.

Table 3. Maternal body weight gain (g/rat±SD) for F_0 generation females assigned to caesarean-sectioning and natural delivery[a]

Interval	Dose in mg/kg/day (# of Dams)[b]			
	0 (50)	40 (50)	125 (50)	400 (50)
Pretreatment Days 0-6, (c-sect.and natural delivery)	31.8±7.8	32.1±8.8	30.2±8.7	31.1±11.8
Treatment Days 6-16, (c-sect.and natural delivery)	55:6±13.2	58.1±14.2	53.0±13.2	44.0±15.2**
Posttreat. C-Sectioning Days 16-20	66.4±17.4	67.7±10.7	71.9±12.3	70.7±20.1
C-Sectioning Overall Days 0-20	156.4±27.0	155.3±19.2	156.5±22.8	144.6±27.2
Natural Delivery Treatment Days 16-20	50.4±15.4	55.8±11.3	49.0±14.6	46.3±16.3
Natural Delivery Overall Days 0-20	135.0±30.5	148.0±22.6	130.4±24.8	122.6±24.9

a Data extracted from the study report Tables B4 and C4, pages 79 and 174.
b Number of dams was 18-24 for c-sectioning rats and 21-25/treatment group for natural delivery rats for overall intervals and days 16-20
** Statistically significant $p \leq 0.01$

3. Feed Consumption - Feed consumption data are summarized in Table 4. Mean feed consumption values for rats in the 400 mg/kg/day dose groups were not affected prior to dosing but statistically significantly ($p \leq 0.01$) depressed (13.7%) during the common 10 day dosing period (days 6-16). Mean feed consumption values over the gestation days 0-20 were depressed at this dose level by 8.2%, ($p \leq 0.05$) and 8.8%, ($p \leq 0.01$) for the rats assigned to c-section and natural delivery, respectively. On treatment days 6-9, rats in the 125 mg/kg/day dose group also exhibited a slight (1.3%), but statistically significant ($p \leq 0.01$) depression in mean feed consumption values. High dose group rats assigned to natural delivery also had reduced feed consumption values on days 16-20 and 0-20 of gestation and on days 8-12 of lactation.

Table 4. Maternal feed consumption (g/rat/day) for females assigned to caesarean-sectioning and natural delivery[a]

Interval	Dose in mg/kg/day (# of Dams)[b]			
	0 (47)	40 (43)	125 (45)	400 (46)
Pretreatment Days 0-6, c-sect. and natural del.	23.3±2.2	23.1±1.9	23.1±2.3	23.0±2.1
Treatment Days 6-9, c-sect. and natural del.	22.0±3.0	20.6±2.6	19.2±2.6**	16.2±4.0**
Treatment Days 6-16, c-sec. and natural del.	22.6±2.3	22.5±2.4	21.4±2.6	19.5±2.6**
C-Sectioning Post-treatment Days 16-20	28.8±3.2	29.3±3.7	31.1±4.4	27.8±4.8
C-Sectioning Overall Gestation Days 0-20	24.3±2.2	24.4±2.3	24.0±2.2	22.3±2.1*
Natural Del. Treatment Days 16-20	23.6±3.3	23.3±3.3	22.2±4.3	20.9±3.4
Natural Del. Overall Gestation Days 0-20	22.8±2.0	22.6±1.8	21.9±2.4	20.8±1.7**

a Data extracted from the study report Tables B5 and C7, pages 80 and 178.
b Number of dams/treatment group was 18-24 in the c-section groups and 21-25 in the natural delivery groups for the overall intervals and days 16-20
* Statistically significant $p \leq 0.05$
** Statistically significant $p \leq 0.01$

4. Gross and Histologic Pathology - There were no treatment-related gross pathologic findings upon necropsy.

   There were no treatment related morphometric or histologic changes in the F1 generation adults.

5. C-Section Data - Observations for those dams designated for c-sectioning are summarized in Table 5. The mean fetal weights for the males from the dams administered 400 mg/kg/day test article were slightly (4.6%), but statistically significantly depressed. The pregnancy rates, maternal wastage, numbers of corpora lutea and implantations, total litters, (viable and dead), resorptions (early and late), sex ratios, and implantation losses (pre and post) were similar between control and treated groups. In addition, there were no statistically significant differences in any of the treated groups compared to the controls in the mean weights of the female fetuses.

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Table 5. Caesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	40	125	400
Rats Assigned (Mated)(#)	25	25	25	25
Rats Pregnant(#) Pregnancy Rate (%)	24 (96)	18** (72**)	24 (96)	22 (88)
Rats Nonpreg.(#)	1	7	1	3
Maternal Wastage # Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	 0 0 0 0 0	 0 0 0 0 0	 0 0 0 0 0	0 1 1 0 0 0
Total Corpora Lutea(# CL) CL/Dam(#±SD)	427 17.8±2.4	328 18.2±3.1	436 18.2±3.0	407 19.4±2.3
Total Implant.(#) Implant./Delivered Litter(#±SD)	375 15.6±3.2	298 16.6±3.8	395 16.4±3.1	362 17.2±2.5
Tot.Litters(#)	24	18	24	21
Tot.Live Fetuses(#) Live Fetuses/Dam(#±SD)	342 14.2±3.6	275 15.3±3.7	371 15.4±2.9	338 16.1±3.0
Dead Fetuses(#) Dead Fetuses/Dam	0 0	0 0	0 0	0 0
Resorptions(#) Early(#) Late(#) Resorptions/Dam(#) Early(#) Late(#) Litters With Total Resorptions(#) Dams With Any Resorptions(N/%)	33 32 1 1.38 1.33 0.04 0 17/70.8	23 23 0 1.28 1.28 0 0 10/55.6	24 24 0 1.00 1.00 0 0 17/70.8	24 24 0 1.14 1.14 0 0 14/66.7
Sex Ratio (% Males±SD)	49.2±15.5	48.8±17.0	51.4±11.5	45.9±18.1
Pre-implantation Loss (%)[b]	12.2	9.1	9.4	11.1
Post-implantation Loss (%)[b]	8.8	7.7	6.1	6.6
Mean Fetal Weight (g±SD) Males Females	 3.67±0.21 3.40±0.20	 3.63±0.21 3.45±0.27	 3.69±0.28 3.48±0.21	 3.50±0.26* 3.30±0.29

a Data extracted from the study report Tables B7, B8 and B18, pages 82, 83 and 139-142

b Calculated by reviewer using the following formulas: pre-implantation loss = number of corpora lutea - number of implantations/number of corpora lutea x 100; post-implantation loss = number of implantations - number of live fetuses/number of implantations x 100

** Statistically significant $p \leq 0.01$; * $p \leq 0.05$

6. Natural Delivery Data - Observations for those dams designated for natural delivery are summarized in Table 6 and litter data are summarized in Table 7. The pregnancy rates, duration of gestation and parturition, maternal wastage, numbers of implantations, total litters, (viable and dead), viability and lactation indices, sex ratios, and live pups per litter during days 1-22 of lactation (postpartum) were similar between control and treated groups. However, mean body weights for the pups from the 400 mg/kg/day group F_0 dams assigned to natural delivery were statistically significantly ($p \leq 0.01$) decreased on days 5 preculling (9.3%), day 5 postculling (11.7%) and day 22 (7.3%) postpartum, respectively.

Table 6. Natural delivery observations (Dams)

Observation	Dose (mg/kg/day)			
	0	40	125	400
Rats Mated(#)	25	25	25	25
Rats Pregnant(#) Pregnancy Rate(%)	23 (92)	25 (100)	21 (84)	24 (96)
Nonpregnant(#)	2	0	4	1
Duration Gestation (Days ± SD)	23±0.4	23.±0.3	23±0.4	23±0.4
Duration Parturition (Days ± SD)	1.1±0.3	1.0±0.0	1.0±0.0	1.0±0.0
# Aborted # Premature Delivery	0 0	0 0	0 0	0 0
Implantations/Del.Litter (#±SD)	16.2±3.2	17.5±2.0	15.4±2.9	16.8±1.8
Total Litters(#)	22	25	21	24
Live Fetuses(#) Live Fetuses/Dam(#±SD)	321 14.6±2.8	406 16.2±2.0*	288 13.7±3.7	349 14.5±3.4
Dead Fetuses(#) Dead Fet./Dam(#±SD)	4 0.2±0.5	6 0.2±0.5	5 0.2±0.5	8 0.3±1.0
Viability Index (N/N) (%) [b]	313/321 97.5	395/406 97.3	283/288 98.3	334/349 95.7
Lactation Index (N/N) (%) [c]	147/147 100.0	180/180 100	140/140 100	156/156 100

a Data extracted from study report Tables C11 and C12, pages 182-184.
b Number of live pups on day 5 (pre-culling) postpartum/number of liveborn pups on day 1 postpartum.
c Number of live pups on day 22 (weaning) postpartum/number of live pups on day 5 (postculling) postpartum.

Table 7. Natural delivery observations (Litters)[a]

Observation	Dose (mg/kg/day)			
	0	40	125	400
Live Pups/Litter(#±SD) Day 1 Day 5, preculling Day 5, postculling Day 22	 14.4±2.8 14.2±2.9 7.9±0.4 7.0±0.0	 16.0±2.1 15.8±2.2 8.0±0.0 7.2±0.4	 13.6±3.7 13.5±3.6 7.7±1.3 7.0±0.0	 14.3±3.3 13.9±3.3 7.7±1.0 7.1±0.3
Mean Pup Weight (g±SD) Day 1 Day 5, preculling Day 5, postculling Day 22	 6.4±0.6 10.8±1.4 11.1±1.1 60.5±3.9	 6.2±0.6 10.2±1.0 10.6±1.0 58.6±4.2	 6.5±0.5 10.8±1.2 11.1±1.2 59.5±3.2	 6.2±0.4 9.8±1.4** 9.8±1.0** 56.1±3.8**
Sex Ratio (Males) (%±SD)	55.2±12.1	51.1±12.5	50.8±17.9	52.4±11.8

a Data extracted from study report Table C12, pages 184-188
** Statistically significant p≤0.01

F. DEVELOPMENTAL TOXICITY

1. C-Section Pups: Pup examinations included external, visceral, and skeletal observations at necropsy. There were no statistically significant differences in any of the treatment groups compared to the controls in the number or percent of fetuses with any alteration or in the percent of fetuses with any alteration per litter (Table 8).

Table 8. Summary of total affected fetuses delivered by c-section.[a]

Observations	Dose in mg/kg/day			
	0	40	125	400
#Fetuses (#litters) examined	342 (24)	275 (18)	371 (24)	338 (21)
Fetuses With Any Alterations (#/%)	10/2.9	11/4.0	15/4.0	9/2.7
Fetuses with Any Alterations/Litter(%±SD)	2.63±4.89	3.84±4.90	3.97±9.38	2.53±4.05

a Data extracted from study report Table B9, page 84.
* Statistically significant p≤0.05

External Examination - There were no treatment-related external findings in any of the dose groups. The most common findings are presented in Table 9.

Table 9. Fetal external alterations[a]

Observations	Dose (mg/kg/day)			
	0	40	125	400
#Fetuses (#litters) examined	342 (24)	275 (18)	371 (24)	338 (21)
Alterations				
Eye, Bulge, depressed Litter Inci. N(%) Fetal Inci. N(%)	 0 (0) 0 (0)	 0 (0) 0 (0)	 1 (4.2) 1 (0.3)	 0 (0) 0 (0)
Body, anasarca Litter Inci. N(%) Fetal Inci. N(%)	 1 (4.2) 1 (0.3)	 0 (0) 0 (0)	 1 (4.2) 1 (0.3)	 1 (4.8) 1 (0.3)

a Data extracted from study report Table B10, page 85

Fetal Soft tissue alterations - There were no treatment-related fetal soft tissue alterations observed at any dose level. The most common findings are presented in Table 10.

Table 10. Fetal soft tissue alterations[a]

Observations	Dose (mg/kg/day)			
	0	40	125	400
#Fetuses (#litters) examined	164 (23)	135 (18)	177 (24)	164 (21)
#Fetuses (#litters) affected	1 (1)	1 (1)	0 (0)	1 (1)
Alterations				
Heart, Situs Inversus Litter Inci. N(%) Fetal Inci. N(%)	1 (4.3) 1 (0.3)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)
Kidneys, Dilation of Pelvis Litter Inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	1 (5.6) 1 (0.7)	0 (0) 0 (0)	1 (4.8) 1 (0.6)

a Data extracted from study report Table B11, page 86

Skeletal Alterations - There were no treatment-related skeletal alterations observed at any dose level. The most common skeletal findings are listed in Table 11

Table 11. Fetal skeletal alterations[a]

Observations	Dose (mg/kg/day)			
	0	40	125	400
#Fetuses (#litters) examined	178 (24)	140 (18)	194 (24)	174 (21)
Alterations [b]				
Skull, Small eye socket, Litter Incidence N(%) Fetal Incidence N(%)	0 (0) 0 (0)	0 (0) 0 (0)	1 (4.2) (0.5)	0 (0) 0 (0)
Thoracic vert. centrum, unilat. ossification, Litter Inci. N(%) Fetal Inci. N(%)	1 (4.2) 1 (0.6)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)
Thoracic Vert., Centrum Bifid, Litter Inci. N(%) Fetal Inci. N(%)	1 (4.2) 1 (0.6)	1 (5.6) 1 (0.7)	0 (0) 0 (0)	0 (0) 0 (0)
Ribs, (Summary of Completely Ossified and wavy Ribs) Litter Inci. N(%) Fetal Inci. N(%)	2 (8.3) 2 (1.1)	2 (11.1) 2 (1.4)	3 (12.5) 10 (5.2)**	1 (4.8) 1 (0.6)
Sternebra, (Summary of Incompletely Ossified and Unossified) Litter Inci. N(%) Fetal Inci. N(%)	1 (4.2) 2 (1.1)	0 (0) 0 (0)	2 (8.3) 2 (1.0)	3 (14.3) 3 (1.7)
Pelvis, (Summary of Incompletely Ossified Pubes, Ischia and Ilia) Litter Inci. N(%) Fetal Inci. N(%)	1 (4.2) 2 (1.1)	3 (16.7) 5 (3.6)	2 (8.3) 3 (1.5)	2 (9.5) 1 (1.1)

a Data extracted from the study report Table B12, pages 87-89.
b # Fetuses (#litters)
** Statistically significant $p \leq 0.01$

2. Natural Delivery Pups: There were no gross pathologic fetal findings at necropsy for pups naturally delivered and sacrificed on days 5, 12, and 22 postpartum (Table 12).

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Table 12. Pup gross alterations[a]

Observations	Dose (mg/kg/day)			
	0	40	125	400
#Pups (#litters) Examined	187 (22)	245 (25)	164 (21)	202 (24)
Alterations				
Small Kidneys Litter Inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	1 (4.2) 1 (0.5)
White Material in Urinary Bladder Litter Inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	0 (0) 0 (0)	1 (4.8) 1 (0.6)	0 (0) 0 (0)
Distended Urinary Bladder Litter inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	1 (4.0) 1 (0.4)	0 (0) 0 (0)	0 (0) 0 (0)
Fluid in Abdominal Cavity Litter Inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	1 (4.0) 1 (0.4)	0 (0) 0 (0)	0 (0) 0 (0)
Distended/Dilated Uterus With Clear Fluid, Litter Inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	2 (8.0) 2 (0.8)	0 (0) 0 (0)	0 (0) 0 (0)
Mottled Liver Litter Inci. N(%) Fetal Inci. N(%)	1 (4.5) 1 (0.5)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)
Dial. Renal Pelvis Litter Inci. N(%) Fetal Inci. N(%)	0 (0) 0 (0)	1 (4.0) 1 (0.4)	0 (0) 0 (0)	0 (0) 0 (0)

a Extracted from study report Table C15, pages 202-203

Body weight data for the pups delivered naturally are presented in Table 13. In the 400 mg/kg/day dose group, males exhibited slight (4.4-8.0%), but statistically significant ($p \leq 0.01$ and 0.05) decreases in mean body weights on days 1-22 postweaning and female pups exhibited a slight (8.7%, $p \leq 0.01$), but statistically significant decrease in mean body weights on day 1 postweaning. However by day 29 postweaning, mean body weights for both sexes were no longer significantly different from the control group rats.

Male pups in the 40 and 125 mg/kg/day dose groups also exhibited slight (4.4-4.6%), but statistically significant ($p \leq 0.05$) decreases in mean body weights on day 1 postweaning. These means, while statistically significant, were not considered biologically significant because of a combination of their small magnitudes, lack of persistence beyond Day 1 and lack of other indications of toxicity in pups at these dose levels. There were no treatment-related changes in feed consumption at any dose level in the F_1 pups during days 1-71 post-weaning.

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Table 13. Body weights (g/rat±SD) of naturally delivered F1 generation pups (Days 1-71 Postweaning)[a]

Day Postweaning	Dose in mg/kg/day (# of Pups/Sex)			
	0 (63)	40 (75)	125 (59)	400 (66)
MALES				
Day 1	65.4±6.8	62.5±6.1*	62.4±6.0*	60.2±6.2**
Day 8	113.8±11.0	111.6±10.1	111.8±8.9	106.6±12.4**
Day 22	238.3±20.3	235.1±18.7	234.3±18.5	227.9±20.4**
Day 71	505.1±65.0	513.5±47.7	495.7±57.8	499.4±48.8
FEMALES				
Day 1	62.3±6.7	62.5±7.6	63.3±7.3	56.9±5.5**
Day 8	101.5±11.6	101.3±10.3	102.4±9.9	98.3±9.6
Day 22	177.5±16.4	180.3±15.1	181.2±15.0	174.2±13.4
Day 71	299.9±38.3	309.6±29.2	302.5±25.4	295.2±40.3

a Data extracted from study report Tables D9 and D11, pages 436 and 438
* Statistically significant $p \leq 0.05$
** Statistically significant $p \leq 0.01$

Pups in the 400 mg/kg/day treatment group also exhibited a slight (13.2%, $p \leq 0.01$), but statistically significant increase in brain/body weight ratios on day 12 postpartum. The increase in this ratio was due to the significantly reduced (12.4%, $p \leq 0.01$) terminal body weights in this group. The weights of the various parts of the brain (telencephalon, diencephalon/mesencephalon, medulla oblongata/pons and cerebellum) for pups in this group necropsied on days 74-93 were not significantly affected (Table 14).

There were no treatment related morphometric or histologic changes in the pups.

Table 14. Brain weights of naturally delivered F_1 generation pups[a]

Part of Brain[b]	Dose in mg/kg/day			
	0	40	125	400
MALES				
Telencephalon (g±SD)	1.28±0.06	1.26±0.11	1.31±0.07	1.23±0.08
Diencephalon/Mesencephalon (g±SD)	0.32±0.03	0.31±0.05	0.31±0.04	0.31±0.02
Medulla Oblongata/Pons (g±SD)	0.24±0.05	0.23±0.04	0.23±0.06	0.24±0.07
Cerebellum (g±SD)	0.30±0.05	0.31±0.03	0.30±0.06	0.29±0.06
Total Brain (g±SD)	2.15±0.10	2.14±0.12	2.11±0.07	2.07±0.12
FEMALES				
Telencephalon (g±SD)	1.13±0.06	1.21±0.09	1.21±0.08	1.17±0.06
Diencephalon/Mesencephalon (g±SD)	0.29±0.03	0.30±0.04	0.30±0.04	0.31±0.04
Medulla Oblongata/Pons (g±SD)	0.23±0.04	0.23±0.04	0.24±0.09	0.23±0.02
Cerebellum (g±SD)	0.27±0.06	0.29±0.02	0.28±0.07	0.29±0.02
Total Brain (g±SD)	1.92±0.09	1.99±0.06	2.01±0.16	1.99±0.08
BOTH SEXES				
Brain Weights (g±SD)	1.37±0.09	1.39±0.07	1.41±0.08	1.35±0.11
Brain/Body Weight (%)	4.84±0.36	5.12±0.43	5.14±0.45	5.48±0.59**

a Data extracted from study report Tables C16, D5, and D8, pages 204, 432, and 435
b Combined sex data were obtained at day 12 postpartum
** Statistically significant $p \leq 0.01$

There was no mortality or significant differences in clinical signs of toxicity, mean feed consumption values, indicators of sexual maturity (weights of left or right epididymis or testis, or the days for which the testes descended, the prepuce was observed to be separated or the vagina was patent), or neurotoxicity parameters of the high dose F_1 pups from the F_0 dams allowed to deliver naturally. Selected data for these findings are summarized in Tables 15-19.

Table 15. Sexual maturation parameters (Days±SD) for pups from dams assigned to natural delivery[a]

Parameter	Dose in mg/kg/day (# of Males/Females)			
	0 (63/63)	40 (75/75)	125 (59/60)	400 (66/66)
Testes Descended	23.1±0.3	23.0±0.2	23.1±0.4	23.1±0.3
Weight, Left/Right Epididymis (g±SD)	0.6±0.07/ 0.62±0.07	0.61±0.06/ 0.63±0.14	0.60±0.07/ 0.62±0.16	0.60±0.07/ 0.61±0.06
Weight, Left/Right Testis (g±SD)	1.80±0.14/ 1.82±0.19	1.77±0.21/ 1.80±0.16	1.72±0.20/ 1.76±0.14	1.79±0.16/ 1.79±0.14
Preputial Separation	49.4±3.5	49.1±4.5	48.8±2.0	50.0±2.6
Vagina Patent	32.0±1.5	31.6±2.0	32.0±1.7	32.3±1.5

a Data extracted from study report Table D17, page 444

Auditory startle habituation was unaffected by treatment at all dose levels (Table 16).

Table 16. Acoustic startle responses (Peak Amplitude ±SD) for pups from dams assigned to natural delivery[a]

Day Postweaning	Dose in mg/kg/day (# of Rats/Sex)			
	0 (21)	40 (25)	125 (20)	400 (22)
MALES				
Day 21-25	39.9±17.8	35.4±16.3	35.4±16.2	35.2±14.7
Day 59-63	79.5±51.0	79.6±43.5	78.2±39.6	85.1±49.3
FEMALES				
Day 21-25	43.2±16.9	34.5±17.1	32.8±12.2	32.0±15.9
Day 59-63	59.6±19.3	60.0±39.8	60.8±19.4	50.2±21.3

a Data extracted from study report Table D18, page 445 and 446; data are the average of 50 trials (10 blocks) measured between days 21-25 (5 blocks) and 59-63 (5 blocks) postpartum.

In a passive avoidance paradigm and a watermaze swim task, learning, short-term retention, long-term retention and/or response inhibition in the male and female offspring were evaluated. There were no treatment-related effects on these parameters (Tables 17 and 18). The statistically significant reduction in the watermaze retention latency trial in the 125 mg/kg/day group on day 2 was considered unrelated to treatment by the study investigators because the value was not dosage-dependent, and an increase in this parameter, rather than a reduction, is considered to be an adverse effect.

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Table 17. Passive avoidance performance (Number of Seconds±SD) for pups from dams assigned to natural delivery[a]

Criteria	Dose in mg/kg/day (# of Rats Tested)			
	0 (21)	40 (25)	125 (20)	400 (22)
Learning, Males Day 1[b]				
Trials to Criterion	5.6±2.1	5.6±2.6	5.4±2.5	5.5±2.8
Latency Trial 1	8.0±10.4	5.1±4.3	5.8±4.2	4.7±2.5
Latency Trial 2	23.6±21.4	22.8±16.8	27.2±21.0	25.3±20.4
Failed to Learn	0	0	0	0
Learning, Females Day 1[b]				
Trials to Criterion	4.1±0.6	4.7±1.1	4.5±1.1	4.1±0.9
Latency Trial 1	6.0±3.4	5.9±3.6	6.8±5.2	6.4±4.2
Latency Trial 2	30.6±20.3	24.0±19.1	26.2±22.3	39.6±20.7
Failed to Learn	0	0	0	0
Retention, Males Day 2[b]				
Trials to Criterion	2.8±0.6	3.2±2.5	3.4±1.7	3.0±1.0
Latency Trial 1	23.4±23.9	29.4±24.1	25.4±23.3	23.4±23.4
Retention, Females Day 2[b]				
Trials to Criterion	2.7±0.6	2.8±0.7	2.6±0.6	2.8±1.0
Latency Trial 1	34.3±26.1	30.3±23.5	33.2±26.3	36.9±24.1

a Data extracted from study report Table D19, page 447
b Days 1 (learning phase) and 2 (retention phase) of testing were separated by a one-week interval.

Table 18. Watermaze performance (Number of Seconds±SD) for pups from dams assigned to natural delivery[a]

Criteria	Dose in mg/kg/day (# of Rats Tested)			
	0 (21)	40 (25)	125 (20)	400 (22)
Learning, Males Day 1[b]				
Total Trials	9.2±3.3	10.0±3.0	9.2±2.7	8.9±2.9
Errors/Trial	0.4±0.2	0.4±0.1	0.5±0.3	0.4±0.2
Latency Trial	14.1±7.8	18.6±10.6	15.0±12.6	13.0±7.6
Failed to Learn	0	0	0	0
Learning, Females Day 1[b]				
Total Trials	8.6±2.3	9.4±2.5	9.8±3.0	8.6±2.8
Errors/Trial	0.4±0.2	0.4±0.2	0.4±0.1	0.4±0.2
Latency Trial	13.7±7.4	15.6±8.2	14.2±9.4	15.3±7.4
Failed to Learn	0	0	0	0
Retention, Males Day 2[b]				
Total Trials	6.7±2.1	8.2±2.1	6.4±2.5	6.5±1.9
Errors/Trial	0.2±0.2	0.2±0.2	0.1±0.1	0.1±0.1
Latency Trial	13.8±12.7	10.4±5.5	7.9±4.2*	9.4±4.6
Retention, Females Day 2[b]				
Total Trials	7.0±2.6	6.7±2.2	7.7±2.9	6.4±2.0
Errors/Trial	0.2±0.3	0.2±0.2	0.2±0.2	0.1±0.1
Latency Trial	10.7±6.9	10.4±5.1	13.0±13.4	11.7±6.8

a Data extracted from study report Table D21, page 452
b Days 1 (learning phase) and 2 (retention phase) of testing were separated by a one week interval.
* Statistically significant $p \leq 0.05$ using parametric tests, not significant using nonparametric tests

Motor activity measurements for F_1 generation pups from dams assigned to natural delivery are summarized in Table 19. While the total mean values for both numbers of movements and time spent in movements for all treatment groups at all test periods showed no effects, male pups in the 400 mg/kg/day dose group tested on day 18 postpartum exhibited minimal, but statistically significant increases both the number of movements and the time spent in movement in several 5 minute test blocks (3/18 blocks for number of movements and 2/18 blocks for time spent in movement). In addition at day 18 postpartum, in all 18 blocks for males and in 14 of 18 blocks for females, time and number of movements were increased in the high-dose as compared to the controls, although the differences were statistically significant only at the blocks noted above for males (see Attachment 1). On day 14, the time and number of movements were increased for females at all dose levels compared to the controls (total mean values and in 16-17 out of 18 blocks), however, the differences were not statistically significant. Motor activity measurements at days 22 and 61 were comparable to the controls at all dose levels. The increases in motor activity at days 14 and 18 are considered transient indications of neurotoxicity.

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Table 19. Motor activity measurements for pups from dams assigned to natural delivery[a]

Sex, Day Postpartum	Dose in mg/kg/day			
	0	40	125	400
Males, Day 14				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	308.6±300.2	250.4±220.9	292.0±234.8	315.9±261.9
Time Spent in Movement (Sec.±SD)	614.6±685.5	474.4±485.7	538.4±490.0	612.0±585.6
Females, Day 14				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	253.4±211.4	341.0±289.8	328.2±280.9	302.6±169.7
Time Spent in Movement (Sec.±SD)	485.5±462.7	687.8±687.4	669.5±667.7	585.0±384.7
Males, Day 18				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	750.2±392.6	801.3±391.0	872.0±404.0	1035.5[b]±254.0
Time Spent in Movement (Sec.±SD)	1692±959.3	1745.5±899.0	1918.0±946.1	2284.0[b]±572.1
Females, Day 18				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	688.2±396.2	805.5±319.4	782.5±398.9	815.3±396.5
Time Spent in Movement (Sec.±SD)	1504.7±891.8	1804.7±754.8	1738.8±992.3	1825.0±939.3
Males, Day 22				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	593.8±314.0	604.7±216.0	609.5±221.7	667.8±276.7
Time Spent in Movement (Sec.±SD)	1297.8±716.0	1317.8±497.2	1339.8±512.1	1452.8±650.8
Females, Day 22				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	625.4±242.6	595.5±224.2	707.0±225.5	618.5±277.8
Time Spent in Movement (Sec.±SD)	1333.1±563.0	1274.8±493.4	1544.4±491.5	1338.1±616.9
Males, Day 61				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	928.8±301.5	953.5±284.2	851.2±243.9	856.6±241.6
Time Spent in Movement (Sec.±SD)	2131.8±715.4	2204.4±699.9	1952.0±534.5	1976.0±553.7
Females, Day 61				
Pups Tested(#)	21	25	20	22
Movement(#±SD)	928.9±237.5	958.5±286.1	956.2±225.7	954.7±254.3
Time Spent in Movement (Sec.±SD)	2130.1±588.0	2194.8±669.4	2206.4±534.8	2184.4±609.6

a Data extracted from study report Tables C13 and D20 pages 189-200 and 448-451; values are totals of 18 blocks.
b Statistically significant increases were seen in 3/18 and 2/18 5-minute test periods for number of movements and time spent in movement, respectively. Total mean values were not statistically significant.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS The study report concluded that oral administration (gavage) of N-methylneo-decanamide to rats at a level of 125 mg/kg/day from days 6-15 of gestation and from gestation day 6 through day 11 of lactation caused clinical observations and reduced maternal feed consumption. At a dose of 400 mg/kg/day, maternal rats had reduced body weights and body weight gains and pups had reduced male fetal body weight in caesarean-delivered fetuses, postpartum and postweaning F_1 generation body weights, increased brain/body weight on day 12 postpartum and minimally affected motor activity on day 18 postpartum. The maternal NOAEL is 40

mg/kg/day and the developmental NOAEL is 125 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: N-Methylneodecanamide (95.9% pure) was administered continuously via gavage to 50 female rats/dose at levels of 0, 40, 125, or 400 mg/kg/day on days 6 through 15 of presumed gestation for developmental toxicity evaluations (pups delivered by c-sectioning) or on days 6 of presumed gestation through day 11 of lactation (postpartum) for developmental neurotoxicity evaluations (pups delivered naturally).

   Dams in the 400 mg/kg/day dose group exhibited treatment-related mortality, statistically significant ($p \leq 0.01$) increases in the incidence of clinical signs of toxicity (excess salivation, rales, ataxia, urine stained fur and decreased motor activity), statistically significant reductions in the mean body weights on gestation days 9-16 (3.1-5.0% decreases, $p \leq 0.01$ or 0.05), a 20.9% decrease ($p \leq 0.01$) in body weight gain for gestational days 6-16 (body weight gain increased over days 0-22 of lactation), decreases in feed consumption during gestation days 6-20 (13.7% decrease, $p \leq 0.01$) and 0-20 (8.2%-8,8%, $p \leq 0.05$) and days 8-12 of lactation (8.2% decrease, $p \leq 0.01$).

   At the 125 mg/kg/day dose level, maternal toxicity was characterized by treatment-related clinical signs (excessive salivation and rales) and slight (1.3%), but statistically significant ($p \leq 0.01$) reductions in feed consumption during the common treatment interval (days 6-9 of gestation).

   The statistically significant decrease in the pregnancy rate in the 40 mg/kg/day treatment group (Table 5 of this DER and Table B7, page 82 of the study report) was not considered to be related to test article administration because pregnancy was established before the test article was first administered (day 6 of gestation), the event was not dose-related and the total number of pregnancies in the low- and high- dose groups were comparable to the total number of pregnancies in the control group rats.

   Maternal NOAEL = 40 mg/kg/day
   Maternal LOAEL = 125 mg/kg/day

2. DEVELOPMENTAL TOXICITY: Developmental toxicity was noted by a 4.6% statistically significant reduction in mean body weights in caesarean-derived male fetuses from dams administered 400 mg/kg/day test article from days 6-15 of gestation, a 7.3-11.7% statistically significant reduction in mean body weights in the naturally delivered F_1 generation pups on days 5-22 of lactation and a 13.2% increase in brain/body weights on day 12 postpartum from dams administered 400 mg/kg/day test article from day 6 of gestation through day 11 of lactation (postpartum). The reduced body weights were greater in males than females. Although the lactational body weights were reported as combined for both sexes, the post-weaning body weights were especially depressed in the high dose males. These pup body weight effects persisted long after the treatment was discontinued in the dams, although feed consumption in all dose groups was comparable to the controls during post-weaning. The statistically significant increase in the fetal incidence of delayed rib ossification (wavy ribs) in

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the 125 mg/kg/day dose group (Table 11 of this DER) was not considered to be treatment related because there was no indication of a dose response relationship for this finding in any of the other dose groups.

Developmental NOAEL = 125 mg/kg/day
Developmental LOAEL = 400 mg/kg/day

3. Neurotoxicity: Neurotoxicity was suggested by minimally, but statistically significant affected motor activity on day 18 postpartum in male pups from dams administered 400 mg/kg/day test article from day 6 of gestation through day 11 of lactation (3/18 blocks for number and 2/18 blocks for time spent in movement). In addition at day 18 postpartum, in all 18 blocks for males and in 14 of 18 blocks for females, time and number of movements were increased in the high-dose as compared to the controls, although the differences were statistically significant only at the blocks noted above for males (see Attachment 1). On day 14, the time and number of movements were increased for females at all dose levels compared to the controls (totals and in 16 to 17 blocks out of 18), however, the differences were not statistically significant. Motor activity measurements at days 22 and 61 were comparable to the controls at all dose levels. The increases in motor activity at days 14 and 18 are considered a transient indication of neurotoxicity.

Neurotoxicity NOAEL = 125 mg/kg/day
Neurotoxicity LOAEL = 400 mg/kg/day (transient effect)

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C. STUDY DEFICIENCIES Actual data of concentration analyses were not reported; only a summary table is presented. No methodology was indicated. Analytical data presenting the purity of the test substance and the actual concentration of the dosing formulations were not provided.

Although this deficiency is not expected to alter the conclusions of the study, the registrant should 1) explain the discrepancy between the purity listed in this MRID (95.9%) as compared to that listed for this same study as reported in MRID 44211902 (95.8%) and the reproduction study with the same lot (96.08%); and 2) provide acceptable analytical data to confirm the purity of the test material and the actual concentrations of the dosing formulations.

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ATTACHMENTS

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