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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - rat §83-6; OPPTS 870.6300
DP BARCODE: D229252
P.C. CODE: 041402
TEST MATERIAL (PURITY): none; diet restriction
CHEMICAL: S-ethyl hexahydro-1H-azepine-1-carbothioate
SYNONYMS: Ordram

Molinate

SUBMISSION CODE: S510660
TOX CHEMICAL NO.: 444

CITATION: Allen, S. L. (1995). Molinate: Developmental Neurotoxicity Study in Rats Using Diet Restriction. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study NO. RR0638/F0 and RR0638/F1, December 1, 1995. MRID 44064705. Unpublished.

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: This was a special developmental neurotoxicity study (MRID 44064705) , designed to generate historical control data for several endpoints in the Alpk:APfSD rats. Three dosing regimens [control diet *ad libitum* plus daily gavage of sterile water, *ad libitum* diet for 6 hours [meal-fed], 80% restriction] were compared to control diet *ad libitum*. Thirty female Alpk:APfSD rats/dietary regimen were administered their diet from gestation day 2 through lactation day 11.

F0 Segment Survival and clinical signs were comparable among the groups. Daily gavage during pregnancy and lactation had no apparent adverse effects on clinical signs, body weight, food consumption, or reproductive performance, and the resultant pups were indistinguishable from pups of untreated control rats. Both means of dietary restriction [meal fed and 80% restriction] resulted in decreased dam body weights [85%-89% of control] and body-weight gains [overall 60% and 75% of control, respectively] during gestation, with the meal-fed dams displaying the largest decrease. Neither the number of pups born nor pup body weight at birth was affected by dietary restriction, but the meal-fed group displayed the lowest percentage of litters with all pups born live [79% *vs* 92%-100%]. In the meal-fed group, a slightly smaller litter size [11.3 *vs* 12.3] and a slightly lower mean pup body weight [5.2 *vs* 5.5 grams], which resulted in a statistically significant decrease in total litter weight [58.5 *vs* 66.6 grams], was observed compared to the control diet group.

F1 Segment Day 12 body weights of the pups were decreased [82%-84% of the control] for both sexes in the meal-fed and 80% restriction diet groups compared to the control diet group, but both groups and sexes displayed comparable body weights to the control group by study end, having had access to feed *ad libitum* from day 12 *post partum*. Another difference observed among the groups was a shortening of the time to vaginal opening, which occurred in the meal-fed and 80% restriction groups compared to the control diet group. Female pups in the meal-fed group displayed a statistically-significant decrease in brain weight compared to the control diet group females on day 63. There was no clear or consistent difference observed among the groups in any of the neurotoxicity parameters monitored [motor activity, learning and memory tests, auditory startle test], and brain length/width measurements were comparable among the groups.

Findings include (a) a shortening of the time to vaginal opening in the female offspring of the dams on both the 80% restriction and the meal-fed dietary regimens, and (b) decreased brain weight in the female offspring of the meal-fed dams at day 63 *post partum*.

This developmental neurotoxicity study in the Alpk:APfSD rat is classified acceptable/guideline. It was performed to (1) generate historical control data on the Alpk:APfSD rat, (2) assess the effect of daily oral gavage on pregnant female rats during the intervals of gestation days 7-29 and lactation days 1-11, (3) assess the effects of dietary restriction in the form of (a) an 80% restricted diet and (b) meal fed during a 6-hour period, and (4) demonstrate the testing facility's competence in evaluating effects in neonatal rats. It also satisfies the guideline requirement for a developmental neurotoxicity study (OPPTS 870.6300) in the rat.

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

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: none; assessing effects of diet restriction

2. Test animals: Species: rat
Strain: Alpk:APfSD
Age at mating: ≈11 weeks of age
Weight at mating: 211-302 g on arrival
Source: Barriered Animal Breeding Unit, Biological Services Section, Alderley Park, Macclesfield, Cheshire, UK
Housing: mated females were housed in individually cages on multiple rat racks. From gestation day 15 until litters were weaned, the dams were provided with solid cage floors and bedding material.
Diet: stock CT1 diet supplied by Special Diet Services Limited, Stepfield, Witham, Essex, UK ad libitum [except for restricted group]
Water: mains water by automatic system ad libitum
Environmental conditions: standard laboratory
Acclimation period: females were from laboratory breeding unit; 16 females supplied on each of seven days, plus 8 females on the 8th day, over an 18-day period.

B. PROCEDURES AND STUDY DESIGN

In life treatment dates – September, 1993 to December, 1993.

Mating: Virgin female rats were paired overnight [at Breeding Unit] with males of the same strain. Vaginal smears were taken the following morning. The day when spermatozoa were detected was designated day 1 of gestation, and the female was delivered to the experimental unit on that day.

3. Animal assignment and dosing: There were thirty replicates [randomized blocks], each containing one rat from each group. Cages within each replicate were assigned to one of four groups using computer-generated, random number permutations. Each rat was allocated randomly to a cage within the replicate. There was no statement as to whether any females that were mated with the same male were distributed across the groups. The individual rat numbers for each cage were determined by group and replicate, and each rat was individually identified with its allocation number by ear punch. There was no indication of whether the groups were standardized with respect to body weight at study start. There were 30 dams/group, and the dams were fed the test diets from day 7 of gestation through day 11 of lactation. One group received the control diet *ad libitum*, one group also received control diet *ad libitum* plus a gavage dose of sterile water once daily from day 7 of gestation through day 11 of lactation [1 mL/100 g body weight, based on dosing day's body weight], one group received *ad libitum* diet for 6 hours per day [fed meal], and one group received ≈80% of an *ad libitum* diet. It is assumed that stock diet was provided [on arrival] from gestation

days 1 to 6 and from lactation day 12 until study termination [lactation day 29].

4. Dose selection rationale: The level of dietary restriction [≈80% of normal] was chosen to reflect maximum reduction in food consumption that is likely to be observed in studies of this type, based on historical data from reproduction studies performed at the testing facility. The purpose of the study was to investigate the effects of dietary restriction during pregnancy and lactation on the postnatal development of the resulting offspring. Additionally, one group received a daily oral dose of water from gestation day 7 through lactation day 11 *via* gavage to simulate the dosing procedure used in the developmental toxicity studies.

5. Diet preparation and analysis

Not applicable.

C. OBSERVATIONS

NOTE: The study was divided into two segments: F0 segment investigated maternal [e.g., body weight, food consumption] effects, reproductive performance, and observations in resulting offspring up to day 5 *post partum*. On day 5 *post partum*, offspring were selected for the F1 segment, which investigated developmental neurotoxicity for individual pups.

1. Maternal [F0] observations - All rats were examined on arrival to ensure that they were healthy and that they exhibited normal activity. **Clinical observations** Detailed clinical observations were monitored daily and included (1) an assessment of signs of autonomic function [e.g., lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis]; (2) description, incidence, and severity of any convulsions, tremors, abnormal motor function, abnormal behavior, etc.; (3) changes in level of arousal; (4) alterations in respiration. **Body weight** Body weight of each dam was recorded on arrival [day 1], on days 4, 8, 15, and 22 of gestation and on days 7, 14, 22, and 29 of lactation. The body weights of the group receiving water *via* gavage were recorded on the days of dosing also [gestation day 7 through lactation day 11]. **Food consumption** The amount of food consumed daily by each dam was determined by providing a weighed quantity of food in a glass jar to each dam from day 2 of gestation until day 11 of lactation and calculating the amount consumed from the residue on subsequent days.

Each litter was examined daily, and any dead pups up to and including day 18 post partum were discarded. Any abnormal pups up to and including day 18 post partum were killed and discarded. A count of all pups [dead and alive] was made within 24 hours of parturition [day 1] and on day 5 post partum. The sex of the pups was determined at these same times. All pups were weighed on days 1 and 5 post partum, and the data were presented by litter and sex.

Pathology of dams - Those dams whose litters were not selected [as well as those observed to have parturition difficulties and females that did not litter by assumed gestation day 25] were exsanguinated under terminal anaesthesia with halothane Ph.Eur. [FLUOTHANE] vapour and subjected to a gross post mortem

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examination [including an examination of the uterus for the presence of implantation sites]. At study termination [on or after day 29 *post partum*, after their litters had been weaned], the dams were sacrificed and subjected to a gross post mortem examination.

2. Selection of F1 animals - Litters were culled to 8 pups [4/sex] on day 5 post partum such that sexes were represented equally [selection procedure not indicated]. NOTE: It was stated that litters containing less than 3 pups/sex were not selected for use on the F1 study. The procedure was designed such that the pups retained in each litter were selected at random [with no preference to larger pups, etc.] from those available of each sex. There was no attempt to standardize litters with less than 8 pups at day 5 post partum. Pups [including whole litters] not selected at day 5 post partum for the F1 study were sacrificed by dislocation of the neck and discarded. Once the selected pups had been identified individually [on day 5 post partum, pups were identified individually within the litter by foot tatoo (# included dam and pup number); from day 12 post partum, pup were identified outside the litter by tatooing the tail with the dam number], they were transferred to Study No. RR0638/F1, each being allocated to the same group as its parent dam. Although remaining with the dam, once pups had been selected, each was allocated an individual identity number and a cage number. Two cage numbers were allocated to each litter so that once weaned and separated by sex, cage numbering did not change.

3. F1 offspring evaluations – Each rat was observed at least once daily, with any changes in behavior or clinical condition being recorded at the same time that each was weighed. Detailed clinical observations [same as those described for the dams above] were recorded [time frame of these was not stated], but presumably at the same time the rats were weighed and prior to each behavioral test [i.e., **motor activity** (days 14, 18, 22, and 60); **tests for learning** (days 21 and 59); **test for memory** (days 24 and 62); **auditory startle test** (days 23 and 61] for those pups selected for that test only.

Body weight: Bodyweights were recorded for the F1 pups on post partum days 5, 12, 18, 22, and then weekly from weaning [day 29] to day 57 and prior to sacrifice [day 63].

Litters were removed from their dams on day 29 post partum and housed by sex on separate racks. The male and female cages on the different racks were housed in the same position as the dams [i.e., group positions on the racks remained the same throughout the F0 and F1 segments of the study].

Developmental landmarks - The age of the pups when vaginal opening [females checked daily from day 29] and preputial separation [males checked daily from day 43] occurred was determined.

Motor activity An automated activity recording apparatus was used to monitor locomotor activity. One male and one female from each litter was assigned to this test on day 5, and the same pups were tested on study days 14, 18, 22, and 60. Each observation period was divided into 10 scans of five-minute duration. Treatment groups were counter balanced across cage/device numbers [up to 32 rats/trail/run], and each rat was returned to the same activity monitor for each trial.

An auditory startle habituation test [using an automated recording apparatus] was performed on one male

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and one female pup/litter [assigned to test on study day 5], and these rats were tested on study days 23 and 61. The mean response amplitude and time to maximum amplitude on each block of 10 trials [5 blocks of 10 trials/session on each day of testing] was measured. No other details were provided. In the developmental neurotoxicity study on Molinate [MRID 44079201] it was stated that " After an acclimation period of 5 minutes during which a background noise level [~35 dB] was maintained, each rat received at total of 50 trials [~110 dB for 40 msec, with 10-second inter-trial-interval and a 100 msec recording window]. " It is assumed that a similar procedure was followed in the current study, since the data generated here are to be compared to the developmental neurotoxicity study data.

Associative learning and memory were assessed for one male and one female pup per litter [assigned on study day 5] using a water Y-maze with only one escape ladder. For each trial, the time required to find the escape ladder was recorded. Each pup was subjected to 6 water maze trials on study days 21 and 59 in order to assess learning ability. Three days after learning had been tested, the pups were subjected to a further 6 water maze trials per day on study days 24 and 62 in order to assess memory. The position of the escape ladder was different for the trials conducted at days 21-24 and days 59-62. A straight "maze" [channel] was used to evaluate swimming ability. Each rat completed one trial in the straight channel immediately after the 6 trials in the water maze.

Pathology of F1 offspring: Dead pups up to and including day 18 post partum were discarded. Any abnormal pups from day 1 to 18 post partum, including those not selected at day 5 post partum, were sacrificed and discarded. Pups on, or after day 19 *post partum* were sacrificed *via* over exposure to halothane Ph. Eur. Vapor followed by exsanguination and subjected to a gross necropsy. **Scheduled termination: Study day 12** At least 10 rats/sex/group [not more than one male or one female per litter] were sacrificed by exposure to CO_2 in rising concentrations, and the brain was immediately removed, weighed, the length and width measured, and immersion-fixed in 10% neutral buffered formol saline. **NOTE: This differs from the procedure used in MRID 44079201, in which the brain was immediately exposed and immersion-fixed in 10% neutral buffered formol saline, and after an interval of at least 24 hours, the brain was removed, weighed, and the length and width were measured.** The rats were subjected to a gross examination [necropsy], and any abnormal tissues were fixed [as above] and stored. The brains from rats in the *ad libitum* diet group and those in the 80% diet restriction group were processed for histopathological examination [brain embedded in paraffin wax, sectioned in the transverse plane at 7 levels, and stained routinely with hematoxylin and eosin]. All sections were examined by light microscopy. **Study day 63** At least 10 rats/sex/group [not more that 1 male or 1 female per litter] were processed as above except that the brain was removed immediately without fixing and, after weighing and measuring the length and width, was discarded. An additional 8 rats/sex/group [one male or one female per litter] were anaesthetized deeply with a lethal i.p. dose of barbiturate and sacrificed by perfusion fixation with modified Karnovsy's fixative. The rats were perfused with a volume of fixative approximately equivalent to their estimated body weight. The brain was removed, weighed, and the length and width were measured. Tissues saved were: brain [not further defined here; in MRID 44079201, the brain tissues saved included forebrain, cerebrum, midbrain, cerebellum, pons and medulla oblongata], cervical and lumbar region of the spinal cord, Gasserian ganglia, dorsal root ganglia [including the cervical and lumbar regions], spinal roots, gastrocnemius muscle, sciatic

nerve, sural nerve, tibial nerve, and macroscopically abnormal tissue. The remaining rats were sacrificed and discarded.

The brain and gastrocnemius muscle were embedded in paraffin wax, sectioned, and stained with H & E. Additionally, sections of spinal cord from the lumbar and cervical region with dorsal root ganglia and spinal roots attached were decalcified and embedded in paraffin wax, sectioned, and routinely stained with H & E. The remaining tissues were embedded in Araldite, and semi-thin sections were cut and stained with toluidine blue.

Histopathological examination: **Study day 12** The brains from rats in the control diet group and the 80%-restricted diet group were examined in the transverse plane at 7 levels. Neuropathological examination did not include morphometric analysis. **Study day 63** The brains from 8 rats/sex from the control diet and 80%-restricted diet groups were examined in the transverse plane at 7 levels. Other submitted tissues from these two groups were examined as follows: Spinal cord from the cervical region [C3-C6] and from the lumbar region [L1-L4] were examined in the transverse plane. Spinal roots and dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the Gasserian ganglia from the trigeminal nerve. Transverse and longitudinal sections of the sciatic nerve and transverse sections of the sural and tibial nerves were examined. Sections of the gastrocnemius muscle were examined in the transverse plane. Neuropathology examination did not included morphometric analysis.

Morphometric analysis No morphometric analysis was performed in this study, but MRID 44064704 provided an assessment of the development of the brain of rats at different ages from postnatal day 7 to postnatal day 63.

D. DATA ANALYSIS

1. Statistical analyses: Body weights during gestation and lactation: analysis of covariance on initial [day 1 of gestation] body weight. Food consumption during gestation and lactation: analysis of variance. Mean observed day for preputial separation and vaginal opening, day 1 litter size, day 1 mean pup body weight, and day 1 total litter weight: analysis of variance. The proportion of males with preputial separation observed on days 43, 44, 45, 46, and >46 and the proportion of females with vaginal opening observed on days 32, 33, 34, 35, and >35 in each group: Fisher's Exact Test. For live born pups: **percentages** analysis of variance following the double arcsine transformation of Freeman and Turkey [1950]; **proportion of pups born live and the proportion of litters with all pups born live** Fisher's Exact Test. From day 5 of lactation, cage mean pup body weights: analysis of variance, separately by sex. Motor activity measurements and the maximum amplitude and time to maximum amplitude in the startle response test: analysis of variance, separately by sex. For water maze data, time taken to complete the straight channel: analysis of variance, separately be sex. For Y-maze data, % of successful trials {calculated separately for each rat [criterion for a successful trial was a time less than a cut-off value (cut-off values of 6 seconds were used for day 21 and day 24 pups and 4 seconds for day 59-62 adults)]; analysis of variance following the double arcsine transformation of Freeman and Turkey, separately by sex and for each cut-off value. Brain weight,

brain length, and brain width: analysis of variance, separately by sex.

All analyses were carried out in SAS [1989]. For the Fisher's Exact Tests, the proportions in each group were compared to the control diet proportion. Analyses of variance and covariance for parental body weight and food consumption allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control diet group were provided by the difference between each treatment group least-squares mean and the control least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with that of the control using a Student's t-test, based on the error mean square in the analysis. All statistics were two-sided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: One control diet dam displayed adverse clinical signs indicative of a difficult parturition [hunched, pale, piloerection, sides pinched in, subdued, and vaginal bleeding] and was sacrificed on day 24. There were no clinical signs reported that could be associated with any of the feeding regimens.

2. Body Weight/Body-Weight Gain - **Gestation**: Comparable body weights/body-weight gains were observed between the control diet group and the control diet group administered water *via* gavage. Both the "meal-fed" [85%-92% of control] and the 80% restricted diet group [89%-96% of control] displayed a significantly decreased body weight compared to the control diet group from gestation day 8 on [Table 1]. The meal-fed group also displayed a significant decrease in body weight at gestation day 4 [92% of control]. The meal-fed group displayed a negative body-weight gain during days 1-4 but a gain that was comparable to the control diet gain during days 4-8. The overall body-weight gain for the meal-fed [60% of control] and 80% restricted [75% of control] groups were decreased compared to the control diet group. **Lactation**: Body weight was decreased significantly in the meal-fed [82% of control] and 80% restricted [86% of control] groups on day 7 of lactation and throughout lactation, but the dams in these two groups appeared to recover following receipt of an *ad libitum* diet from day 12 on. By day 29, body weight for these groups was 94% and 93% of the control, respectively. Throughout lactation, body-weight gain for both the meal-fed and 80% restricted diet groups was greater than that of the control diet groups. Mean body weight and body weight gain for control diet + water gavage group were comparable to the control diet group during lactation.

Table 1. Body Weight and Body-Weight Gain •[grams] - F0 Dams				
Period/Day/Diet	Control diet	Control - H20	Meal fed	80% restriction
BODY WEIGHT				
Gestation				
1	254.1±17.8	259.5±9.5	254.6±20.1	247.2±21.9
4	269.1 ±17.9	275.9±9.9	248.1**±24.5 [92]♪	258.5±21.2 [96]
8	282.1±17.9	291.0 ±10.0	260.3**±20.3 [92]	269.8**±21.5 [96]
15	320.8±19.8	328.2 ±13.6	273.2**±21.9 [85]	284.0**±22.4 [89]
22	388.3±26.7	396.3±22.8	335.1**±22.1 [86]	348.3**±27.5 [90]
BODY WEIGHT				
Lactation				
7	317.4±22.7	322.4±17.6	261.5**±16.2 [82]	271.8**±16.7 [86]
14	341.9±23.6	343.2±15.1	303.4**±19.4 [89]	300.1**±14.9 [88]
22	347.2±22.5	350.5±17.7	323.9**±20.5 [93]	319.7**±16.8 [92]
29	331.8±22.7	336.0±17.0	311.1**±17.8 [94]	309.5**±18.3 [93]
BODY-WEIGHT GAIN♬				
Gestation				
days 1-4	15.0	16.4	-6.5	11.3 [75]
days 4-8	13.0	15.1	12.2	11.3 [87]
days 1-8	28.0	31.5	5.7 [20]♪	22.6 [81]
days 8-15	38.7	37.2	12.9 [33]	14.2 [37]
days 15-22	67.5	68.1	61.9 [92]	64.3 [95]
days 1-22	134.2	136.8	80.5 [60]	101.1 [75]
BODY-WEIGHT GAIN♬				
Lactation				
days 7-14	24.5	20.8	41.9	28.3
days 14-22	5.3	7.3	20.5	19.6
days 22-29	-15.4	-14.5	-12.8	-10.2
days 7-29	14.4	13.6	49.6	37.7
day 7-22	29.8	28.1	63.4	47.9

♪ [% of control diet group];
data from Table 3 [gestation] and Table 4 [lactation], pages 48-49 of the report;
♬ calculated by reviewer using data from Tables 3-4

3. Food Consumption - Food consumption [Table 2] was decreased in both restricted feeding groups, as expected, with the meal-fed group displaying the greater decrease, throughout most of the study. In the 80% restriction group [fed ≈22 g/rat/day], the decrease in food consumption varied from 78% to 92% of control during gestation and 55% to 95% of control during lactation compared to the control diet group. All groups consumed less than their normal amount on day 22 of gestation, with the control diet eating the least. The meal-fed dams varied considerably also, consuming from 10% to 92% of the amount consumed by the control diet dams during gestation and from 67% to 81% of control during lactation.

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Table 2. Food Consumption [grams/rat/day] - F0 Dams				
Period, Day, Diet	Control Diet	Control Diet + H20	Meal Fed	80% Restriction
FOOD CONSUMPTION **Gestation**				
2	23.0	22.7	2.4** [10]♪	20.2**[88]
3	23.4	24.3	10.0**[43]	21.3**[91]
4	23.9	24.5	13.3**[56]	21.6** [90]
6	25.2	25.9	16.3**[65]	21.8** [87]
7	25.1	27.0*[107]	17.9**[71]	21.8** [87]
8	26.4	27.5	17.3**[66]	21.9** [83]
10	27.7	28.5	18.7**[68]	22.0** [79]
15	24.0	25.0	22.0**[92]	22.0** [92]
20	27.8	28.0	22.4**[81]	21.7** [78]
22	9.2	13.6	17.7**[192]	15.8** [172]
FOOD CONSUMPTION **Lactation**				
1	13.0	14.7	10.5 [81]	12.3 [95]
2	26.1	28.0	17.4**[67]	23.9 [92]
3	32.5	35.2*	25.0**[77]	30.0*[92]
4	38.3	39.9	28.2**[74]	31.8**[83]
11	58.2	56.8	41.5**[71]	32.0**[55]

♪ [% of control; * p<0.05; ** p<0.01; data from Table 5 [gestation] and Table 68 [lactation], pages 50-53 of the report

4. Reproductive performance - Each diet group [30/group] had at least 2 females that were not pregnant [Table 3], with the restricted diet group and the control diet group displaying the lower number of pregnant dams [26] compared to the control diet + water and meal-fed groups [28]. One control diet litter was born dead. The total number of pups born, litter size, and the number of pups born live/dead were comparable among the groups. The mean number of total pups born and the mean number of live pups born were comparable also. The meal-fed group displayed the lowest percent of litters with all pups born live compared to the other groups [79% *vs* 92%, 92% and 100%].

The ratios of male pups to female pups [not provided in the report] was variable among the groups [51% (control diet), 55% (control diet + water), 57% (meal fed), and 44% (80% restriction)].

Parameter/Diet [ppm]	Table 3. Litter Data			
	control diet	control diet+H20	meal fed	80% restriction
Number Pregnant	26	28	28	26
Number Litters Born Dead	1	0	0	0
Mean Gestation Length (days)	NP	NP	NP	NP
# Litters w/ All Pups Born Live	23/26 [92]♪	26/28 [92]	22/28 [79]	26/26 [100]
Total Pups Born	322	337	322	326
# Pups Born Live	319	333	316	326
Mean Number of Pups/Litter	12.3±3.4	11.9±3.3	11.3±2.4	12.5±3.1
Sex Ratio (% males)●	51	55	57	44

Data from Table 7 [page 54] of the study report; ♪ {%}; NP not provided
● calculated by reviewer using data from Tables 7 & 8 [pages 54-56 of the report

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Macroscopic findings in dams: There were no treatment-related findings.

B. PUP OBSERVATIONS

1. Clinical observations and survival **F1 Litters**: There were no apparent differences in either survival or clinical signs among the groups.

2. Pup Weight and Weight Gain - **F1 Pups** Day 1 *post partum* mean pup body weights [Table 4] were comparable among the groups [on a litter basis; weights for each sex on day 1 were not provided].

Table 4. Body-Weight Data for F1 Pups on Day 1 *post partum* [grams]				
Sex/Diet	control diet	control diet=H2O	meal fed	80% restriction
Mean pup	5.5±0.6	5.7±0.6	5.2±0.5	5.4±0.5
total litter wt.	66.6±16.8	67.1±17.0	58.5*±10.2	66.8±13.9

* p<0.05; data from Table 7 [page 54] of the report

Decreased body weights [Table 5] were observed in both sexes in the 80% restricted diet [83% of control] and meal-fed [84% of control] diet groups beginning at day 12 *post partum*. Statistically-significant decreases in body weight were observed in females through day 43 in the restricted diet group and through day 22 in the meal-fed diet group, with the magnitude of the decrease declining with time. In males, statistically-significant decreases were observed through day 63 in both the restricted and meal-fed diet groups, with the decrease also declining with time. For both sexes in these two groups, body weights were comparable [98% of control] to the control diets by day 63. The control diet + water gavage groups were comparable to the control diet groups throughout the study. Body-weight gains overall [days 5 through 63] were comparable among the groups for both sexes, but initially [days 5-12], the restricted [70% of control] and meal-fed [73% of control] diet groups [both sexes] displayed decreased in body-weight gains. **NOTE**: Test diets were terminated at day 12 and all groups were provided feed *ad libitum*.

Table 5. Body Weight and Body-Weight Gain [grams] - F1 Pups				
Sex/Day/Diet	control diet	control diet+H2O	meal-fed	80% restriction
BODY WEIGHT				
Females				
5	7.6±0.9	8.1±0.8	7.6±0.8	7.9±0.9
12	20.0±2.5	20.4±2.1	16.7** ±1.6[84]	16.6** ±1.3[83]
18	34.3±3.9	34.1±2.7	31.7** ±2.1[92]	31.2** ±1.9[91]
22	47.4±5.2	47.6±3.7	44.8* ±2.8[95]	44.1** ±2.9[93]
29	83.6±8.6	84.9±5.2	80.6 ±4.3[96]	79.8* ±5.1[95]
36	123.3±10.5	124.4±7.1	118.7±6.5	117.9* ±7.1[96]
43	155.3±11.5	156.3±8.3	150.4±8.8	149.1* ±9.3[96]
50.	176.6±13.8	178.4±9.1	173.2±9.7	172.7±10.2
57	197.1±15.6	200.0±10.9	191.7±11.8	192.3±11.7
63	205.7±15.2	208.7±12.3	201.4 [98]±13.3	201.4 ±10.8[98]

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Table 5. Body Weight and Body-Weight Gain [grams] - F1 Pups				
Sex/Day/Diet	control diet	control diet+H2O	meal-fed	80% restriction
BODY WEIGHT				
Males				
5	8.1±0.7	8.4±0.9	8.1±0.8	8.3±0.9
12	21.0±2.3	21.5±2.4	17.5** ±1.6[83]	17.3** ±1.2[82]
18	35.9±3.7	35.8±3.3	33.0** ±1.9[92]	32.2** ±1.9[90]
22	50.1±4.2	50.2±4.5	46.7** ±2.7[93]	45.9** ±3.0[92]
29	90.4±6.8	91.1±6.2	86.0** ±4.1[95]	85.6** ±4.7[95]
36	141.9±9.6	141.8±9.5	135.8* ±5.9[96]	135.4** ±6.9[95]
43	194.9±11.3	195.4±12.1	186.7* ±8.5[96]	186.7* ±10.3[96]
50	251.0±15.0	251.1±14.4	240.6* ±10.7[96]	241.5* ±13.6[96]
57	303.0±17.4	304.1±15.8	291.4* ±10.6[96]	292.6* ±16.0[97]
63	335.4±20.1	338.3±18.1	324.1* ±13.4[97]	324.8* ±17.4[97]
BODY-WEIGHT GAIN♬				
Females				
days 5-12	12.4	12.3	9.1 [73]	8.7 [70]
days 12-18	14.3	13.7	15.0	14.6
days 5-18	26.7	26.0	21.1 [90]	23.3 [87]
days 18-22	13.1	13.5	13.1	12.9
days 22-29	36.2	37.3	35.8	35.7
days 5-29	76.0	76.8	73.0 [96]	71.9 [95]
days 5-63	198.1	200.6	193.8	193.5
days 12-63	185.7	188.3	184.7	184.8
days 29-63	122.1	123.8	120.8	121.6
BODY-WEIGHT GAIN♬				
Males				
days 5-12	12.9	13.1	9.4 [73]	9.0 [70]
days 12-18	14.9	14.3	15.5	14.9
days 5-18	27.8	27.4	24.9 [90]	23.9 [86]
days 18-22	14.2	14.4	13.7 [96]	13.7 [96]
days 22-29	40.3	40.9	39.3	39.7
days 5-29	82.3	82.7	77.9 [95]	77.3 [94]
days 5-63	327.3	329.9	316.0	316.5
days 12-63	314.4	316.8	306.6 [98]	307.5 [98]
days 29-63	245.0	247.2	238.1	239.2

♪ [% of control]; * p<0.05; ** p<0.01; ♬ calculated by reviewer using data from Table 12, pages 65-66 of the report

3. Developmental Landmark Data - There was an acceleration [comparable] in vaginal opening in both the meal-fed [33.7 days] and restricted diet [33.7 days] groups compared to the control diet [34.3 days] and control diet + water groups, and the time of preputial separation was comparable among the dosing regimens [Table 6].

Table 6. Developmental Landmarks of F1 Pups - Age [days]				
Parameter/Diet	control diet	control diet+H20	meal fed	80%
preputial separation - males	44.8±1.2 [73]♪	44.9±1.4 [74]	45.0±1.5 [83]	44.9±1.3 [82]
vaginal opening – females	34.3±1.3 [74]	34.1±1.3 [71]	33.7±1.0** [76]	33.7±0.9** [84]

♪ [n]; ** p<0.01; data from Table 11 [page 64] of the report

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4. Mean Auditory Startle Data - **MALES** There were no clear differences among the groups in either startle amplitude or time to maximum amplitude, although both parameters were increased in all groups on test day 61 *post partum* compared to test day 23 [Table 7]. **FEMALES** No clear differences were observed among the groups for either parameter, although on day 23 *post partum* both the 80% restricted and meal-fed diet group displayed a slight increase in startle amplitude compared to the control and control + water diet groups. Time to maximum amplitude was significantly decreased in the control diet + water and meal-fed groups compared to the control diet group on day 23 *post partum* during the last 3-4 repetitions.

Table 7. Auditory Startle Test - Selected F1 Pups

Sex/Day/Dose	control diet	control diet+H2O	meal-fed	80% restriction
STARTLE AMPLITUDE V				
females n=	22	21	22	24
day 23				
repetition 1-10	226.8±99.9	232.5±84.0	232.4±79.1	306.4±232.2
repetition 11-20	150.1±79.4	151.5±80.6	172.8±59.7	217.8*±126.2
repetition 21-30	126.8±62.3	129.0±84.6	146.2±69.4	161.7±65.0
repetition 31-40	136.1±70.5	123.2±67.3	135.9±65.3	145.1±60.1
repetition 41-50	112.3±66.8	117.2±59.7	140.3±67.0	139.9±63.4
day 61				
repetition 1-10	1017.8±224.1	865.4±264.1	897.9±325.6	946.3±214.1
repetition 11-20	802.4±296.7	695.0±244.7	793.3±363.8	800.5±298.6
repetition 21-30	664.0±230.8	692.2±295.4	673.9±307.1	697.1±302.5
repetition 31-40	634.1±252.9	585.6±186.5	539.7±209.6	713.9±294.1
repetition 41-50	522.8±180.5	542.2±228.0	520.1±219.9	604.1±284.1
STARTLE AMPLITUDE V				
Males	21	21	23	24
day 23				
repetition 1-10	264.9±101.9	287.8±141.3	236.4±117.0	270.8±91.4
repetition 11-20	215.3±75.4	190.5±100.9	175.1±109.2	192.6±64.6
repetition 21-30	160.1±63.4	157.0±86.6	164.0±108.1	162.9±60.6
repetition 31-40	142.5±67.0	163.2±88.5	152.9±98.6	141.1±53.8
repetition 41-50	126.3±54.9	136.1±79.9	137.9±79.7	138.2±62.5
day 61				
repetition 1-10	1151.8±484.5	1108.6±439.9	924.3±331.4	1014.6±372.9
repetition 11-20	882.7±473.8	728.6±297.3	653.7*±317.9	790.9±379.4
repetition 21-30	693.4±352.6	609.6±299.8	669.7±280.0	713.8±361.5
repetition 31-40	587.9±361.8	583.0±329.6	616.0±238.9	679.3±389.8
repetition 41-50	591.6±317.3	556.3±281.5	612.4±247.2	658.3±346.6

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Table 7. Auditory Startle Test - Selected F1 Pups				
Sex/Day/Dose	control diet	control diet+H2O	meal-fed	80% restriction
TIME TO MAXIMUM AMP [MS]				
Females				
day 23				
repetition 1-10	27.3±4.5	28.4±5.6	26.8±4.4	28.2±6.9
repetition 11-20	22.1±4.2	20.9±2.3	19.5*±1.9	22.3±4.3
repetition 21-30	21.2±3.0	19.1*±2.3	19.4*±2.5	20.8±3.4
repetition 31-40	21.7±4.4	19.4*±2.4	19.6*±2.0	20.8±3.7
repetition 41-50	21.4±4.2	18.2*±3.6	18.9*±2.3	19.0*±3.4
day 61				
repetition 1-10	26.2±4.1	26.6±6.3	26.5±4.9	26.2±4.3
repetition 11-20	23.9±4.1	23.9±4.5	24.0±3.6	24.8±3.9
repetition 21-30	23.7±3.7	24.4±3.6	24.1±3.1	23.9±2.1
repetition 31-40	24.0±3.4	25.1±3.8	25.1±2.9	24.1±2.6
repetition 41-50	25.1±3.4	24.2±2.6	25.6±3.9	24.3±4.2
TIME TO MAXIMUM AMP [MS]				
Males				
day 23				
repetition 1-10	26.3±4.5	27.5±3.8	28.8±6.1	27.4±4.9
repetition 11-20	21.4±2.6	21.3±3.1	22.7±3.9	20.8±3.3
repetition 21-30	22.0±3.5	20.6±4.1	21.9±4.2	20.8±3.6
repetition 31-40	20.6±3.7	21.2±3.5	20.5±5.4	20.7±3.4
repetition 41-50	21.4±4.0	21.2±3.6	21.2±4.4	19.5±2.4
day 61				
repetition 1-10	29.4±6.6	29.8±6.0	27.7±4.3	25.9*±4.3
repetition 11-20	25.8±4.5	23.7±3.1	24.8±4.2	24.2±3.5
repetition 21-30	25.9±4.1	25.7±4.3	24.5±2.8	26.0±5.7
repetition 31-40	26.7±3.9	26.3±4.7	24.8±2.8	26.9±4.4
repetition 41-50	25.7±3.5	28.4*±6.1	26.0±4.1	24.0±2.9

[% of control]; * p<0.05; ** p<0.01
data from Tables 15 A&B, pages 80-83 of the report

5. Mean Motor Activity Data – **MALES** Initially [1-5 minutes], the control diet + water group displayed a lower activity level than the other groups on day 14 *post partum*, but all groups displayed a lowering of activity level with time during the 50-minute session [Table 8]. The meal-fed group displayed the lowest overall activity on day 14 *post partum*. On day 18 *post partum*, the meal-fed group continued to displayed the lowest motor activity. Thereafter, comparable levels of activity were observed among the groups. Activity levels increased with age in all groups. **FEMALES** A slightly lower activity level was observed in the control diet + water group on days 14, 18, and 22 *post partum* compared with the other groups. Activity levels increased with age in all groups.

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Table 8. Motor Activity [units not provided]				
Day. Interval/minutes/Sex/Dose	control diet	control diet+H2O	meal fed	80% restriction
day 14				
MALES				
1-5	8.3±14.7	4.0±6.3	7.1±17.8	8.5±13.9
6-10	4.9±13.3	1.8±3.7	3.7±11.9	2.2±4.7
11-15	4.3±9.4	2.8±7.5	1.7±4.0	2.2±4.9
16-20	5.2±13.4	3.5±7.4	0.0*±0.2	3.1±7.9
21-25	2.7±6.5	1.6±3.0	0.3*±1.3	0.6±1.1
26-30	1.2±2.9	1.1±2.8	0.0±0.2	0.6±2.1
31-35	0.9±1.8	1.2±3.0	0.2±0.5	0.3±0.9
36-40	0.7±2.4	0.5±1.6	0.0±0.0	0.3±0.7
41-45	0.5±1.2	0.1±0.5	0.1±0.3	1.0±3.2
46-50	0.7±1.8	0.2±0.9	0.1±0.3	0.5±1.4
overall	29.3±56.8	16.7±22.9	13.3±29.6	19.3±27.1
day 14				
FEMALES				
1-5	10.5±17.4	7.0±11.2	10.4±19.1	9.2±13.7
6-10	7.1±11.7	1.8±4.6	5.5±14.4	5.0±11.1
11-15	3.9±10.1	0.9±1.8	3.3±9.6	1.6±5.5
16-20	3.4±8.3	2.4±5.1	1.8±5.7	2.7±6.7
21-25	2.7±5.9	1.6±3.8	0.7±2.0	3.0±8.4
26-30	0.2±0.7	2.0±6.9	3.5±8.6	0.9±3.2
31-35	0.4±0.7	1.0±2.5	1.1±3.1	2.6±8.1
36-40	0.3±0.9	0.0±0.2	0.7±2.5	0.2±0.7
41-45	0.0±0.2	0.2±0.9	1.5±4.7	0.5±2.4
46-50	0.2±0.8	1.8±6.8	0.8±2.5	1.0±4.1
overall	28.8±40.0	18.8±23.2	29.3±47.4	26.7±44.9
day 18				
MALES				
1-5	17.4±15.4	18.7±19.1	11.7±13.9	17.8±13.8
6-10	21.0±19.4	12.6±14.3	11.3*±16.6	14.9±11.6
11-15	17.7±20.3	13.6±16.6	11.0±18.5	12.7±17.0
16-20	18.7±20.2	6.9*±9.4	5.1**±8.5	10.6±19.1
21-25	14.8±16.9	7.9±12.9	5.3*±7.2	10.5±14.2
26-30	16.1±20.0	8.8±14.0	5.2*±9.6	8.5±13.4
31-35	11.8±15.7	8.4±15.6	5.4±11.6	7.0±12.3
36-40	13.5±16.7	12.7±19.2	6.6±10.0	9.4±15.0
41-45	8.5±17.1	6.7±16.5	5.1±11.7	9.3±15.8
46-50	14.7±23.1	4.3±15.4	7.9±16.3	6.3±14.2
overall	154.2±149.3	100.5±97.0	74.7*±71.5	106.8±85.4

Table 8. Motor Activity [units not provided]				
Day/Interval/minutes/Sex/Dose	control diet	control diet+H2O	meal fed	80% restriction
day 18				
FEMALES				
1-5	20.6±19.0	12.7±17.1	16.3±14.8	18.3±21.3
6-10	19.0±21.1	10.7±12.8	15.2±14.9	16.8±22.0
11-15	15.5±20.0	10.8±14.2	7.8±10.3	14.0±18.0
16-20	14.0±18.1	8.0±14.3	10.7±16.4	11.7±19.0
21-25	13.3±19.8	7.8±11.1	11.8±15.5	11.9±17.8
26-30	10.6±17.6	5.3±7.2	12.0±19.1	12.7±21.9
31-35	8.6±15.4	5.6±11.4	8.6±14.0	11.8±20.8
36-40	11.5±19.7	2.2*±5.7	7.8±13.7	9.5±14.9
41-45	11.5±17.5	1.8*±6.6	8.0±15.1	9.3±17.0
46-50	4.7±11.1	3.7±9.8	5.6±11.4	3.0±8.5
overall	129.2±145.5	68.5±73.8	104.0±95.6	119.1±145.9
Day 22				
MALES				
1-5	36.5±17.0	35.1±16.5	37.1±17.7	35.8±22.8
6-10	34.9±20.6	32.7±19.7	33.6±21.3	24.3±14.5
11-15	21.4±16.8	26.9±17.7	30.6±21.7	23.0±18.5
16-20	23.3±15.3	24.1±18.9	22.6±16.4	19.8±19.1
21-25	13.7±17.7	15.0±15.9	28.9**±18.7	14.4±16.1
26-30	17.3±19.5	14.8±18.5	25.6±19.1	12.6±16.2
31-35	19.6±20.7	17.3±20.7	19.6±17.6	11.8±16.3
36-40	20.1±15.5	22.9±23.7	24.2±20.9	17.0±20.8
41-45	18.8±14.8	16.7±16.6	20.1±18.8	17.2±19.8
46-50	13.3±16.7	10.0±15.5	19.1±16.1	12.4±18.8
overall	219.0±97.9	215.5±119.6	261.4±113.5	188.3±108.7
day 22				
FEMALES				
1-5	38.2±20.6	33.0±19.0	37.9±17.2	32.5±18.3
6-10	38.5±15.3	29.4±20.9	29.8±17.9	28.3±18.1
11-15	33.6±21.0	23.5±22.1	24.3±16.4	26.0±17.1
16-20	22.7±18.0	22.9±19.5	23.8±20.3	25.2±19.0
21-25	27.5±18.6	24.3±23.5	14.6*±13.2	24.9±20.1
26-30	19.2±18.4	22.8±24.9	26.5±19.0	21.5±22.8
31-35	24.3±17.0	18.6±22.7	22.0±18.5	20.4±19.4
36-40	22.6±22.4	19.2±23.8	17.9±19.9	24.8±22.1
41-45	23.7±24.7	11.0±18.7	18.6±20.6	22.7±22.5
46-50	22.7±24.8	21.9±23.8	17.2±20.7	16.4±20.1
overall	273.0±129.1	226.7±141.9	232.5±100.7	242.6±151.6

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Table 8. Motor Activity [units not provided]				
Day/Interval/minutes/Sex/Dose	control diet	control diet+H2O	meal fed	80% restriction
day 60				
MALES				
1-5	56.6±10.1	57.5±8.0	60.0±9.8	55.2±9.1
6-10	60.0±7.6	53.0±11.1	57.4±12.1	53.0*±14.3
11-15	57.3±11.2	52.7±11.7	51.5±16.2	49.4±16.7
16-20	50.5±20.5	45.6±19.6	53.1±18.3	42.3±22.0
21-25	44.4±26.4	38.9±21.7	42.5±23.8	39.8±25.2
26-30	31.1±24.8	35.4±24.0	36.7±25.9	32.8±24.6
31-35	31.8±26.6	24.0±20.4	27.2±24.4	27.2±23.2
36-40	20.3±22.5	17.2±20.9	16.4±21.0	21.2±23.7
41-45	18.3±23.6	15.5±24.8	13.7±23.5	17.5±22.7
46-50	19.0±25.1	13.2±23.1	9.3±19.3	17.2±22.8
overall	389.3±117.8	353.0±114.3	367.9±124.8	355.4±143.3
day 60				
FEMALES				
1-5	48.5±6.5	53.0±6.1	51.5±9.6	50.2±9.4
6-10	51.6±8.1	54.1±8.0	50.4±8.9	52.9±8.9
11-15	55.6±8.3	53.4±8.7	54.1±8.3	55.3±9.8
16-20	55.7±9.1	52.9±11.3	58.0±8.4	54.4±11.4
21-25	52.7±8.8	53.1±14.4	52.2±14.9	48.9±20.0
26-30	52.0±13.5	50.6±17.9	52.7±16.5	48.9±19.9
31-35	57.3±14.4	48.0±21.2	51.6±18.1	46.1±22.3
36-40	50.6±15.2	51.4±22.6	51.1±20.7	43.0±22.6
41-45	52.6±18.5	42.8±21.2	51.2±19.5	43.0±24.1
46-50	49.3±18.8	47.5±21.7	50.3±21.5	41.3±24.6
overall	525.9±71.2	506.9±100.8	523.0±94.0	484.0±107.9

* p<0.05; ** p<0.01; data from Table 13 [pages 67-74] of the report

6. Learning and memory tests – **STRAIGHT CHANNEL:** No clear difference was observed in swimming time among the groups for either sex [Table 9] at any time point. **Y-SHAPED WATER MAZE:** For both sexes, the percent of successful trials [those completed in less than 6 seconds] was increased in the memory phase at both time intervals compared to the learning phase, indicating that learning had occurred [Table 10]. A significant decrease in the percent of successful trials was observed in the day 62 memory phase of the study in females of the control diet + water gavage group compared to the control diet group.

Table 9. Straight Channel Swim [Learning and Memory Tests]				
Sex/Diet/Time [phase]	control diet	control diet + H2O	meal fed	80% restriction
MALES				
Straight channel				
Day 21 [learning]	3.90±1.54	4.74±3.66	3.87±1.38	3.92±1.82
Day 24 [memory]	3.00±1.60	3.64±2.14	2.80±0.61	2.78±1.11
Day 59 [learning]	2.86±0.67	2.79±0.63	3.46±3.25	3.18±1.52
Day 62 [memory]	2.62±0.87	2.60±0.66	2.71±0.75	2.99±1.36

Table 9. Straight Channel Swim [Learning and Memory Tests]

Sex/Diet/Time [phase]	control diet	control diet + H2O	meal fed	80% restriction
FEMALES				
Straight channel				
Day 21 [learning]	3.78±1.61	5.14±4.00	4.16±2.07	4.61±2.86
Day 24 [memory]	2.69±1.22	3.16±0.94	2.59±0.42	2.72±0.84
Day 59 [learning]	2.67±0.92	3.01±2.35	2.89±0.66	2.56±0.29
Day 62 [memory]	2.21±0.56	2.46±0.90	2.63±1.52	2.28±0.44

Data from Table 14, page 75 of the report

Table 10. Water Maze Data [% of successful trials]

Sex/Diet/phase]	control diet	control diet + H2O	meal fed	80% restriction
MALES n=	21	21	23	24
Day 21 [learning]	22.2±20.0	23.0±23.8	24.6±26.5	20.1±18.4
Day 24 [memory]	54.0±22.9	50.8±26.6	62.3±25.7	61.1±21.2
Day 59 [learning]	42.1±23.9	42.9±25.6	45.7±23.1	49.3±23.3
Day 62 [memory]	58.7±29.2	65.9±26.6	59.6±25.8	67.4±23.6
FEMALES n=	22	20	23	24
Day 21 [learning]	21.2±22.5	28.3±24.2	19.6±19.9	20.8±20.8
Day 24 [memory]	66.7±20.6	60.8±24.9	61.6±22.7	59.0±27.6
Day 59 [learning]	45.5±24.2	50.0±25.4	47.8±19.1	48.8±26.8
Day 62 [memory]	78.0±21.4	55.8*±32.6	65.2±28.8	66.7±24.1

* $p<0.05$; data from Table 14, pages 76-79 of the report

C. POSTMORTEM EXAMINATIONS

1. Brain Weight/Length/Width Data – **MALES** Brain weight, length, and width were comparable among the groups at both time points [Table 11]. **FEMALES** The only apparent difference among the groups was noted in the meal-fed female pups who displayed a statistically significant decrease in brain weight at day 63 compared to the control diet group [Table 12].

Table 11. Mean Male Pup Brain Weights (g)*

Group/Day/Parameter	control diet	control diet + H2O	meal fed	80% restricted
Day 12				
Brain Weight	0.98±0.07	1.01±0.06	0.97±0.04	0.95±0.07
Brain Length	19.4±1.2	19.5±1.6	19.0±1.2	19.3±1.2
Brain Width	12.7±0.6	13.2±0.6	13.0±0.4	12.7±0.7
Day 63				
Brain Weight	1.94±0.08	1.95±0.05	1.96±0.07	1.94±0.06
Brain Length	24.3±0.8	24.3±0.9	23.6±0.9	24.2±0.7
Brain Width	14.9±0.5	15.1±0.5	14.8±0.6	14.7±0.5

Data from Table 16 (pages 84 &85) of the report.

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Table 12. Mean Female Pup Brain Weights (g)*				
Group/Day/Parameter	control diet	control diet + H2O	meal fed	80% restricted
Day 12				
Brain Weight	0.94±0.07	0.96±0.07	0.93±0.09	0.95±0.04
Brain Length	19.1±1.1	19.6±1.6	18.5±1.1	19.4±1.2
Brain Width	12.8±0.6	12.8±0.4	12.7±0.6	12.7±0.5
Day 63				
Brain Weight	1.82±0.07	1.84±0.05	1.75*±0.06	1.79±0.07
Brain Length	23.9±1.8	24.0±0.8	23.3±0.9	23.3±1.1
Brain Width	14.6±0.9	14.8±0.6	14.2±0.8	14.7±0.7

Data from Table 16 (pages 84 & 85) of the report.
* Statistically different from controls, $p<0.05$

2. Gross Postmortem Examinations of Pups - Gross postmortem findings for pups sacrificed on Days 12 and 63 were comparable among the groups [both sexes].

3. Histopathological Examinations - There was no evidence of either central or peripheral nervous system developmental abnormalities in either sex.

It was stated that the major feature of the neonatal pup brains [day 12] was the presence of large numbers of mitotic figures and apoptotic-like bodies in all major brain regions, especially in the hippocampal formation, cortex, and olfactory bulb of the forebrain and the superficial granular layer of the cerebellum. Mitotic figures were also seen in the endothelium of the brain capillaries and in the subependymal tissue lining the lateral and third ventricles. This degree of proliferative and regulatory cell death activity was said to be similar in the control diet and 80% restricted diet groups. Also the morphological features of the brain in terms of the presence and spatial relationships of the major brain nuclei were said to be similar in these two groups. No mitotic figures and only an occasional apoptotic body were noted in the brain at day 63. The morphology of the brain of all rats were considered within normal limits at day 63. Nerve fiber degeneration of the sciatic nerve was reported in one male [1 of 8 pups] and two female pups [2 of 8] of the 80% restricted diet group and one female [1 of 8] of the control diet group.

III. DISCUSSION

Three dosing regimens [control diet *ad libitum* plus daily gavage of sterile water, 6-hour meal, 80% restriction] were compared to control diet *ad libitum*. Daily gavage during pregnancy and lactation had no apparent adverse effects on clinical signs, body weight, food consumption, or reproductive performance, and the resultant pups were indistinguishable from pups of untreated control rats. Both means of dietary restriction [meal fed and 80% restriction] resulted in decreased dam body weights and body-weight gains during gestation, with the meal-fed dams displaying the larger decrease. Neither the number of pups born nor pup body weight at birth was affected by either form of dietary restriction, but the meal-fed group displayed the lowest percentage of litters with all pups born live [79% *vs* 100% (80% restricted) and 92% (control diets)]. In the meal-fed group, a slightly smaller litter size [11.3 *vs* 12.3] and a slightly lower mean pup body weight [5.2 *vs* 5.5 grams], which resulted in a statistically significant decrease in total litter weight [58.5 *vs* 66.6 grams] was observed compared to the control diet group. Another difference observed among the groups was a shortening of the time to vaginal opening, which occurred in the meal-

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fed and 80% restriction groups compared to the control diet group. In the 80% restriction group, 44% of the pups were male compared to 51%, 55%, and 57% in the control, control+H2O, and meal-fed groups, respectively. Female pups in the meal-fed group displayed a statistically-significant decrease in brain weight compared to the control diet group females on day 63. There was no clear or consistent difference observed among the groups in any of the neurotoxicity parameters monitored [motor activity, learning and memory tests, auditory startle test and brain length/width measurements].

Discrepancies: In calculating the sex ratio, the reviewer found 12 extra control diet pups and 1 extra meal-fed pup than the number of pups born live listed in Table 7 [page 54] of the report. However, this does not impact negatively on the study results.