[MOLINATE] DEVELOPMENTAL NEUROTOXICITY STUDY §83-6; OPPTS 870.6300

EPA Reviewer: Linda L. Taylor, Ph.D. _____
Reregistration Branch I, HED (7509C)
EPA Secondary Reviewer: Susan Makris, M.S. _____
Toxicology Branch I, HED (7509C)
Branch Senior Scientist: Whang Phang, Ph.D._____
Reregistration Branch I, HED (7509C)

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DATA EVALUATION RECORD

Top Review #012868

STUDY TYPE: Developmental Neurotoxicity Study - rat OPPTS 870.6300

DP BARCODE: D229333
P.C. CODE: 041402
TEST MATERIAL (PURITY): Molinate (96.8% a.i.)
CHEMICAL: S-ethyl hexahydro-1H-azepine-1-carbothioate
SYNONYMS: Ordram

SUBMISSION CODE: S510773
TOX CHEMICAL NO.: 444

CITATION: Horner, S. A. (1996). Molinate: Developmental Neurotoxicity Study in Rats. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study NO. RR0699, June 13, 1996. MRID 44079201. Unpublished.

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 44079201), Molinate [96.8% a.i.] was administered to 30 female Alpk:AP$_f$SD rats/group in the diet at dose levels of 0, 20, 75, and 300 ppm (0, 1.8, 6.9, and 26.1 mg/kg/day, respectively) from gestation day 7 through lactation day 11 [with the day of parturition designated as postnatal day 1].

MATERNAL TOXICITY: There was no evidence of a treatment-related effect on maternal survival or clinical signs of toxicity. Mean maternal body weight values for the 300 ppm group were decreased slightly [93%-94% of control] from day 10 of gestation and throughout lactation [89%-95% of control] compared to the controls. Mean body weight gain at the 300 ppm dose level was decreased prior to dosing [days 1-4 of gestation (88% of control)] and during gestation days 7-22 [76% of control] and 1-22 [80% of control]. During the first 3 days of dosing, dams at the 300 ppm dose level displayed a negative body-weight gain. During lactation, the 300 ppm dose group displayed a negative body-weight gain during days 1-7, and the overall body-weight gain for both the mid- and high-dose groups was decreased [70% and 73% of control, respectively] compared to the control. A statistically significant reduction in group mean food consumption was noted in the 300 ppm group throughout gestation [73%-94% of control] and lactation [75%-87% of control] compared to the control group.

Litter size and the number of pups born live/dead were comparable among the groups, and the mean number of total pups born and live birth index were unaffected by treatment. The mid- and high-dose groups displayed the lowest percent of litters with all pups born live compared to the controls. At 300 ppm, there was an increase in the number of litters with small female pups, a slightly higher mortality rate during days 1 to 5 *post partum*, and the number of missing and presumed dead pups [both sexes] was increased compared to the controls. Whole litter losses occurred at the control [2 litters] and high-dose [4 litters] levels only.

There were no treatment-related findings observed in the dams at necropsy [brain weights were not measured].

SELECTED F1 OFFSPRING: At the high-dose level [300 ppm], there was an increase in preweaning mortality, and a higher number of 300 ppm pups were reported missing/presumed dead compared to the controls. There was a significant decrease in birth weight and an increase in the number of small pups of both sexes at 300 ppm compared to the control group. There was no effect on the sex ratio [percent males].

Decreased body weight was observed at the 300 ppm dose level for both sexes [males 73%-84%/females 72%-82% of control] from day 5-29 of lactation, and the decrease continued post weaning [days 29-63], although the magnitude of the decrease in both sexes [males 81%-88%/females 84%-91% of control] decreased with time. Decreased body-weight gains were observed mainly during the preweaning period in both sexes [64%-84% of control] at 300 ppm.

There was a delay in both preputial separation and vaginal opening at 300 ppm compared to the control groups.

On day 23 *post partum*, there was a significant decrease in the startle amplitude for both sexes at 300 ppm at all 5 intervals, and the females at this time point displayed a dose-related decrease in the startle amplitude, which was statistically significant at all dose levels in 3 of 5 intervals. Males at all dose levels and females at the low- and mid-dose levels displayed comparable responses to those of the controls on day 61, but the high-dose females continued to display a decrease in startle amplitude on day 61. Time to maximum amplitude was increased on day 23 in the high-dose males only and only during the second interval. On day 61, females at 300 ppm displayed a significant increase in the time to maximum amplitude during 4 of the 5 intervals.

Motor activity was comparable among the female groups, but an effect on this parameter cannot be ruled out for males at the 300 ppm dose level because of the initial [day 14] decrease and subsequent, sustained [days 22 and 60 *post partum]*, increase in motor activity observed.

Straight-channel swimming time was increased at 300 ppm in both sexes on day 21 *post partum* compared to the controls but comparable among the groups at all other time points. In both the initial learning [day 21] and memory [day 24] phases of the Y-shaped water maze test, both sexes at 300 ppm had a lower percentage of successful trials compared to the controls throughout the test. In the subsequent learning [day 59] and memory [day 62] phases of the Y-shaped water maze test, comparable successes were observed among the groups [both sexes].

There was a treatment-related decrease in absolute brain weight for both sexes at 300 ppm at both the day 12 and day 63 sacrifice times. Brain length was decreased in both sexes at 300 ppm on day 12, and the females of this group also displayed a decrease in brain width. At day 63, slight decreases in both length and width were observed in both sexes at 300 ppm, but statistical significance was not attained.

There were no treatment-related findings at necropsy on either day 12 or day 63, no microscopic abnormalities in the brains of any pups on day 12, and there were no changes in the central or peripheral nervous systems on day 63 that could be attributed to treatment. With respect to morphometric measurements, treatment–related changes in the cortex and/or cerebellum of the brain [decreased structural measurements and decreased thickness of cellular layers] were observed at the mid- and high-dose levels on day 12, and similar treatment-related changes in the cortex, hippocampus, and/or cerebellum were observed at the 300 ppm dose level on day 63.

The NOEL for maternal toxicity is 75 ppm [6.9 mg/kg/day], and the LOEL for maternal toxicity is 300 ppm [26.1 mg/kg/day], based on decreased body weight/gain and food consumption.

The NOEL for developmental neurotoxicity is 20 ppm [1.8 mg/kg/day], and the developmental LOEL is 75 ppm [6.9 mg/kg/day], based on a reduction in startle amplitude in the auditory startle test in females [day

23] and treatment-related reductions in some morphometric measurements in areas of the cerebellum of the brain [day 12] in both sexes.

At 300 ppm [26.1 mg/kg/day], (1) increased mortality, (2) decreased body weight, (3) a delay in the appearance of developmental landmarks [preputial separation and vaginal opening], (4) an increase in swimming time in the straight channel test at day 21 and reduced performance in the learning and memory tests on days 21 and 24, respectively, (5) a reduction in startle amplitude, (6) an increase in the time to maximum amplitude [days 23 and/or 61], (7) a possible increase [slight] in mean motor activity level in males, (8) reduced brain weight [both sexes on days 12 and 63], brain length [both sexes on day 12], and brain width [females on day 12], and (9) reductions in several morphometric measurements in areas of the cortex, hippocampus, and cerebellum of the brain were observed.

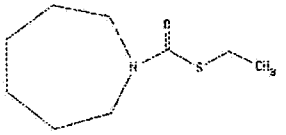
The developmental neurotoxicity study in the rat is classified **Acceptable** and it satisfies the guideline requirement for a developmental neurotoxicity study in the rat (§83-6; OPPTS 870.6300).

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Molinate
Description: amber liquid
Batch #: P4 D7534/25; CTL Reference No. Y06367/025/001
Purity: 96.8% a.i.
CAS #: 002212-67-1
Source: Zeneca Agrochemicals, Jealotts Hill, UK
Storage conditions: ambient temperature, in the dark
Empirical formula: $C_9H_{17}NOS$
Structure:



2. Vehicle: milled diet [CT1]

3. Test animals: Species: rat
Strain: Alpk:AP$_f$SD
Age at mating: not provided [virgin females]
Weight at mating: not provided
Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK
Housing: mated females were housed in individual cages on multiple rat racks; each was transferred to clean cages and racks, as necessary during study. 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
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

1. In life treatment dates - start: July 17, 1995; end: October 26 1995

2. 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 cage per treatment group. Computer-generated, random number permutations were used to allocate the cages within each replicate to an experimental group. Each rat was allocated randomly to a cage, although 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 at dose

levels of 0 ppm, 20 ppm, 75 ppm, and 300 ppm from day 7 of gestation through day 11 of lactation. Stock diet was provided [on arrival] from gestation days 1 to 6 and from lactation day 12 until study termination [lactation day 29]. Stock diet was also provided to those F1 offspring that remained on study postweaning.

4. Dose selection rationale: The study report states that the dose levels used were based on the results of a study [not identified] performed in the Alpk:AP$_f$SD rat at the testing facility. NOTE: The low and high doses are the same as those used in the 2-generation reproduction study [MRID 44403201].

5. Diet preparation and analysis: Premixes were prepared by mixing appropriate amounts of Molinate with 500 grams of milled CT1 diet, and the premixes were then added to 29.5 kg of CT1 diet and mixed thoroughly. The study report does not state how frequently the diets were prepared. Samples of all diets were taken from each batch of diet and analyzed quantitatively for Molinate prior to being administered to the dams. The homogeneity of Molinate in the CT1 diet was determined by analyzing samples from the low- and high-concentration diets, and the chemical stability of Molinate in the diet at 5 ppm and 400 ppm was determined in a concurrent study [RR0674]. All dietary levels prepared during the study were analyzed for concentration [two dates are reported, but it is not clear whether these diets were the only ones prepared].

Results

Homogeneity Analysis: Homogeneity analyses of the low- [20 ppm] and high- [300 ppm] concentration diets demonstrated that the mixing procedures were adequate. The overall mean concentrations of Molinate were within 7% of the nominal concentration [Table 2 (page 53) of the report].

Stability Analysis: mean % of initial concentration [stored at room temperature] for the 5 ppm diet was 100% on Day 0, 88.5% [January, 1995 batch] on Day 23, and 90.4% [April, 1995 batch] on Day 28. For the 400 ppm diet, the mean % of initial concentration [stored at room temperature] was 100% on Day 0, 90.7% [January, 1995 batch] on Day 24, and 91.6% [April, 1995 batch] on Day 28. When stored at -20°C, the mean % of initial concentration was 90.2% on day 23 [January, 1995 batch] and 96% [April, 1995 batch] on day 42 at the 5 ppm nominal concentration. For the 400 ppm concentration stored at -20°C, the % of initial concentration was 96.1% [January, 1995 batch] on day 24 and 98.8% [April, 1995 batch] on day 42 [data from Table 3, pages 54-57 of the report].

Concentration Analysis: The mean achieved concentrations of Molinate in the diets were within 7% of the nominal concentration [Table 1, pages 51-52 of the report].

C. OBSERVATIONS

1. Maternal 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) description and incidence of postural and gait abnormalities; (4) description and incidence of any unusual or abnormal behavior, excessive or repetitive actions, and general signs of toxicity [e.g., thin, dehydrated, altered muscle tone, altered fur appearance, including stains around nose and mouth]. **Body weight** Body weight of each dam was recorded on arrival [day 1], on days 4, 7, 10, 13, 16, 19, and 22 of gestation and on days 1, 4, 7, 10, 13, 16, 19, 22, 25, and 29 of lactation. **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 on days 1, 4, 7, 10, 13, 16, and 19 of gestation and on days 1, 4, 7, 10, 12, 13, 16, 19, 22, and 25 of lactation and calculating the amount consumed from the residue recorded on days 4, 7, 10, 13, 16, 19, and 22 [gestation]/days 4, 7, 10, 12, 13, 16, 19, 22, 25, and 29 [lactation].

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 requiring euthanasia during the study, as well as those whose litters were not selected, were exsanguinated under terminal anaesthesia with halothane Ph.Eur. [FLUOTHANE] vapor and subjected to a gross *post partum* examination [including an examination of the uterus for the presence of implantation sites]. At study termination, the dams whose litters were chosen for the F1 study were sacrificed, as above once their litters had been weaned, and subjected to a gross *post partum* examination on or after day 29 *post partum*.

2. Selection of F1 animals - Litters were culled to 8 pups [4/sex] on day 5 *post partum* such that sexes were represented equally. NOTE: It was stated that litters of 7 or 8 pups with at least 3/sex were 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* [from the write-up on page 20 (Selection of F1 animals), it is not clear whether those litters with less than 8 pups were used; i.e., whether pups in these latter litters were chosen to continue on the study]. 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 *psot partum*, pups were identified individually within the litter by foot tatoo (# included dam and pup number); from day 12 *post partum*, pups were identified outside the litter by tatooing the tail with the dam number], they were transferred to Study No. RR0699/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 - Detailed clinical observations [same as those described for the dams above] were recorded at the same time the rats were weighed and prior to each behavioral test [i.e., **motor activity** (days 14 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: Body weights 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 were 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/trial/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 and one female pup/litter [assigned to test on study day 5], and these rats were tested on study days 23 and 61. After an acclimation period of 5 minutes during which a background noise level [~35 dB] was maintained, each rat received a total of 50 trials [~110 dB for 40 msec, with a 10-second inter-trial interval and a 100 msec recording window]. 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] were measured.

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 pup requiring euthanasia from day 1 to 18 post partum, including those not selected at day 5 post partum, were sacrificed and discarded. **Scheduled termination**: On study *post partum* days 12 and 63, up to 12 rats/sex/group [one male or one female per litter] were removed such that approximately equal numbers of male and female offspring were removed from all litters combined. Additionally, on day 63, 8 rats/sex/group [one male or one female per litter, which included rats that were selected for test procedures] were sacrificed for neuropathological examination. **Study *post partum* day 12** One male or one female per litter were sacrificed by exposure to CO_2 in rising concentrations, and the brain was immediately exposed and immersion fixed in 10% neutral buffered formol saline. After an interval of at least 24 hours, the brain was removed, weighed, and the length [measured from the rostral edge of the olfactory bulb to the cut caudal edge of the brain] and width [measured over the maximum width of the cerebral hemispheres] were measured. The rats were subjected to a gross examination [necropsy], and any abnormal tissues were fixed [as above] and stored. The brains of 6 rats/sex/group of these rats were processed for histopathological examination [brain was embedded in paraffin, sectioned, and stained]. **Study *post partum* day 63** At least 10 rats/sex/group [usually not more than 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 [see above], was discarded. An additional 8 rats/sex/group [one male or one female per litter, which included rats previously subjected to one of the test procedures] 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 at ~100 mm Hg pressure for ~20 minutes. The brain was removed, weighed, and the length and width were measured. Tissues saved were: brain [including forebrain, cerebrum, midbrain, cerebellum, pons and medulla oblongata], cervical and lumbar regions 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.

Histology processing: **Study *post partum* day 12** The brains from 6 rats/sex/group were embedded in paraffin wax, sectioned, and stained routinely with hematoxylin and eosin [H & E]. **Study *post partum* day 63** - All submitted tissues from 8 rats/sex/group from the control and high-dose groups, along with the brains from 8 rats/sex/group from the low- and mid-dose groups, were processed as follows: 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 regions 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 *post partum* day 12** The brains of 6 rats/sex/group were examined in the transverse plane at 7 levels. Neuropathological examination included morphometric analysis. **Study *post partum* day 63** The brains from 8 rats/sex/group were examined in the transverse plane at 7 levels. Other submitted tissues from the control and high-dose rats 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 gastrocnemium muscle were examined in the transverse plane. Neuropathology examination included morphometric analysis.

Morphometric analysis: At scheduled termination [*post partum* day 12 or day 63], the brains were removed, weighed, and the length and width were measured. The cerebellum was removed by cutting the cerebellar peduncles, and the brain and cerebellum were embedded in 3.3% agar, as an aid to accurate trimming. The brain was trimmed into 7 layers [Figure B, appended to File Copy], and processed into paraffin wax blocks [Blocks 1-7], embedded rostral surface down. The cerebellum was also sectioned sagittally in the mid-line and processed into paraffin wax blocks [Blocks 8a-8b] embedded medial surface down. Hematoxylin and eosin slides were prepared from each block for qualitative examination using a light microscope. Those from Blocks 2, 3, 4, 5, and 8a/8b were also used for morphometry and were sectioned such that they represented as much as possible the picture of an 'ideal' level, as shown in Figures C, D, E, F, G [mislabeled E] of the report [copies appended to File Copy]. For morphometry, all measurements were made using a Kontron Image Analyzer. Measurement was made with a Macro set-up or microscope at a magnification of convenient size. Although measurements were made for both left and right sides of the brain, where relevant, the values reported in the summary tables were mean values from both measurements.

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. For live born pups on day 1: **percentages** analysis of variance following the double arcsine transformation of Freeman and Tukey [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 (values of 3, 4, 5, 6, 7, 8, 9, and 10 were used)]}: analysis of variance following the double arcsine transformation of Freeman and Tukey, separately by sex and

for each cut-off value. Brain weight, brain length, brain width, and morphometric measurements: analysis of variance and analysis of covariance on final body weight, separately by sex.

All analyses were carried out in SAS [1989]. For the Fisher's Exact Tests, the proportions in each treated group were compared to the control proportion. Analyses of 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 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.

2. Historical control data: Historical control data [MRID 44064704 (Morphometry), MRID 44064702 (Learning & Memory Assessment), MRID 44064701 (Motor Activity), MRID 44064705 (Dietary Restriction)] were provided to allow comparison with concurrent controls.

3. Positive control data: Data were submitted for the following chemicals (doses): **motor activity**: amphetamine sulphate (0.1 mg/kg i.p.) and chlorpromazine hydrochloride (10 mg/kg i.p.); **learning and memory**: scopolamine (10 mg/kg i.p.). The endpoints evaluated were the same as those in the present study. Motor activity was assessed one hour after dosing the rats at 14, 18, and 22 days of age [single injection] using the Coulbourn Lab Linc Infrared Motion Activity System. No information was found in the study report on developmental neurotoxicity regarding the apparatus used. No data were reported for 60 days of age [a time point in the Molinate developmental neurotoxicity study]. Learning and memory were assessed [10 trials] in a Y-shaped water maze followed by 1 trial in a straight channel on days 1 and 4 following single doses on day 1 and/or day 4.

Motor activity. Amphetamine sulphate tended to increase activity levels in the male pups at all ages tested and in females aged 22 days, and chlorpromazine hydrochloride tended to decrease activity levels in female rat pups at 14 and 18 days of age and in male pups at 22 day of age. **Learning and memory**. For the water "Y" maze time trial results, deficits in the ability to learn a swimming task, as tested by recall of the task 3 days later, were detected and could be differentiated from effects on short-term and reference memory. The straight channel demonstrated that effects were not due to decreases in the ability to swim.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: One control dam, one low-dose dam, and one mid-dose dam [days 23-24] were euthanized because their litters were born dead. There were no treatment-related clinical signs of toxicity at any dose level.

2. Body Weight - **Gestation**: Comparable body weights were observed among the groups through day 7 [first day of dosing]. Thereafter, body weight was significantly reduced [93%-94% of control] in the 300 ppm group during gestation days 10 to 22 [Table 1]. Body-weight gain [calculated by reviewer] was reduced during gestation days 7-22 [76% of control] and overall [80% of control]. Body weights and body-weight gains were comparable among the low-, mid- and control dams throughout gestation. **Lactation**: Body weight was decreased [88%-95% of control] significantly throughout lactation at the high-dose level. The high-dose dams displayed body-weight loss during lactation days 1-4 and days 1-7, and the mid-dose dams

displayed a gain that was 34% of the control value during the same time frame. All Molinate groups displayed a decrease in body-weight gain during days 4-7 of lactation, with the mid-dose dams displaying a loss. Overall, the mid- [70% of control] and high-dose [73% of control] dams displayed a decreased body-weight gain compared to the control. All groups displayed body-weight loss during the day 22-29 interval.

Mean body weight and body weight gain for the low-dose group were comparable to the control group during gestation and lactation. Statistically-significant decreases in body weight during days 19 to 29 of lactation in the 20 ppm dams were not judged to represent an adverse, treatment-related, response due to the general lack of dose response and the minimal nature of the effect [1-4% decrease from control value with no supporting decrease in body-weight gain].

Table 1. Body Weight and Body-Weight Gain [grams] - F0 Dams

Period/Day/Dose	0 ppm	20 ppm	75 ppm	300 ppm
BODY WEIGHT				
Gestation				
1	264.1	262.4	264.3	259.9
4	283.9 [282.3]♦	279.7 [280.0]	282.6 [280.9]	277.3 [280.1]
7	298.9 [297.3]	294.0 [294.3]	297.7 [296.1]	292.3 [295.0]
10	310.9 [309.2]	307.7 [307.8]	308.3 [306.6]	285.9 [288.5]** (93)
13	327.6 [325.8]	322.0 [322.1]	322.2 [320.7]* (98)⊕	302.1 [304.9]** (94)
16	344.0 [342.2]	337.8 [337.8]	335.0 [333.3]** (97)	317.3 [320.0]** (94)
19	379.8 [377.7]	373.6 [373.7]	372.6 [371.2]	350.0 [352.8]** (93)
22	406.6 [404.3]	402.3 [402.6]	404.8 [403.0]	374.1 [377.0]** (93)
BODY WEIGHT				
Lactation				
1	314.8 [312.4]	305.0 [305.7]	314.8 [312.5]	295.5 [297.7]* (95)
4	324.6 [322.4]	314.9 [315.2]	318.1 [315.9]	290.7 [291.8]** (91)
7	330.1 [328.0]	318.3 [317.3]* (97)	315.7 [317.7]* (97)	291.9 [296.3]** (90)
10	341.6 [339.2]	331.2 [330.0]	329.4 [331.7]	299.9 [305.3]** (90)
13	349.5 [347.1]	340.8 [339.6]	334.7 [336.7]* (97)	304.7 [309.9]** (89)
16	357.5 [355.0]	349.6 [348.2]	343.2 [345.3]	316.9 [321.5]** (91)
19	366.9 [364.8]	357.2 [355.4]* (97)	350.8 [352.6]** (97)	325.8 [331.2]** (91)
22	366.9 [364.5]	357.0 [355.7]* (98)	351.2 [353.6]* (97)	333.7 [338.9]** (93)
25	360.8 [358.5]	346.6 [345.0]** (96)	347.0 [349.8]	330.3 [336.2]** (94)
29	354.7 [353.1]	338.8 [337.0]** (99)	340.8 [344.1]	327.6 [334.0]** (95)
BODY-WEIGHT GAIN♪				
Gestation				
days 1-4	19.8	17.3	18.3	17.4
days 4-7	15.0	14.3	15.1	15.0
days 1-7	34.8	31.6	33.4	32.4
days 7-10	12.0	13.7	10.6	-6.4
days 7-22	107.7	108.3	107.1	81.8 (76)
days 1-22	142.5	139.9	140.5	114.2 (80)
BODY-WEIGHT GAIN♪				
Lactation				
days 1-4	9.8	9.9	3.3	-4.8
days 4-7	5.5	3.4	-2.4	1.2
days 1-7	15.3	13.3	0.9	-3.6
days 7-10	11.5	12.9	13.7	8.0 (70)
days 7-22	36.8	38.7	35.5	41.8
days 1-22	52.1	52.0	36.4 (70)	38.2 (73)
days 22-29	-12.2	-18.2	-10.4	-6.1

♦ [adjusted]; ⊕ (% of control); ♪ calculated by reviewer using data from Tables 5-6
Data from Table 5 [gestation] and Table 6 [lactation], pages 62-63 of the report

3. Food Consumption - Food consumption [Table 2] was significantly decreased at the high-dose level throughout gestation [prior to dosing (days 1-4) and from day 7-22 (73%-91% of control)] and lactation [75%-87% of control]. Values were comparable to the control during gestation at the mid-dose level but somewhat lower during lactation days 7-12 and 13-22 [93%-94% of the control]. The low-dose group displayed a decrease in food consumption prior to dosing [94% of control value on days 1-4 of gestation], during the 3 days following the start of dosing [94% of control on days 7-10 of gestation], and during the first 10 days of lactation, although statistical significance was not attained for this latter decrease. The decreases in food consumption at the low- and mid-doses are not considered treatment-related since there was no consistent dose response.

Table 2. Food Consumption [grams/rat/day] - F0 Dams

Period/Day/Dose	0 ppm	20 ppm	75 ppm	300 ppm
FOOD CONSUMPTION **Gestation**				
1-4	25.0	23.5*	24.7	23.6* [94]
4-7	29.0	27.9	29.1	28.6
7-10	30.1	28.2** [94]♦	28.8	22.1** [73]
10-13	31.9	30.9	31.4	28.1** [88]
13-16	33.2	32.3	32.0	30.0** [90]
16-19	33.2	32.9	33.1	30.2** [91]
19-22	30.5	29.7	30.4	25.4** [83]
FOOD CONSUMPTION **Lactation**				
1-4	26.3	24.1	26.5	20.8** [79]
4-7	44.3	42.3	43.2	38.4* [87]
7-10	50.2	47.3	46.9 [93]	38.5** [77]
10-12	60.0	59.5	56.6 [94]	45.2** [75]
12-13	65.7	66.8	66.2	51.9** [79]
13-16	61.8	60.3	57.5* [93]	47.2** [76]
16-19	70.6	70.0	66.8 [95]	58.4** [83]
19-22	80.2	80.3	75.0* [94]	65.2** [81]
22-25	99.8	100.7	96.9	82.0** [82]
25-29	118.9	119.5	114.8	96.5** [81]

♦ [% of control]; * $p<0.05$; ** $p<0.01$; data from Table 7 [gestation] and Table 8 [lactation], pages 65-66 of the report

4. Test material intake - During gestation, the doses of Molinate ingested by the dams during days 7-22 were 1.8, 6.9, and 26.1 mg/kg/day for the 20 ppm, 75 ppm, and 300 ppm groups, respectively. During lactation days 1-12, the doses attained were 2.7, 10.0, and 36.1 mg/kg/day, respectively.

5. Reproductive performance - All dams [30/group] produced litters, but one litter in each of the control, low-dose, and mid-dose groups was born dead. No adverse effects were observed on litter size or the number of pups born live/dead [Table 3]. The mean number of total pups born and the mean live birth index were not affected by treatment. The mid- and high-dose groups displayed the lowest percent of litters with all pups born live compared to the control [79% and 80% *vs* 90%, respectively].

The mean percent of male pups [calculated by reviewer] was comparable to the control values for all the treatment groups.

Parameter/Dose [ppm]	Table 3. Litter Data			
	0	20	75	300
Number Pregnant	30	30	30	30
Number Litters Born Dead	1	1	1	0
Mean Gestation Length (days)	NP	NP	NP	NP
# Litters w/ All Pups Born Live [%]	26/29 [90]	27/29 [93]	23/29 [79]	24/30 [80]
Total Pups Born	356	387	391	391
# Pups Born Live	351	384	383	383
Mean Number of Pups/Litter	12.1±3.6	13.2±2.3	13.2±2.7	12.8±2.8
Pups Dead [day 1]/# litters affectedΔ	5/3	3/2	8/6	8/6
Mean Live Birth Index	98.8	99.3	97.8	97.8
Percent Males [day 1●]	53.3±16.3	51.0±16.2	50.5±13.4	49.9±15.9

Data from Table 9 [page 67] of the study report; NP not provided
● calculated by reviewer using data from Appendix 8, pages 945-968 of the report;
Δ calculated by reviewer from data in Appendix 6, pages 715-722 of the report

6. Gross Postmortem Examination of Maternal Females: There was no evidence of a treatment-related effect on the gross postmortem findings in the females. Brain weights of the dams were not measured.

7. Histopathological Examinations: There was no histopathological examination of tissues from the maternal rats.

B. PUP TOXICITY

1. Clinical observations and survival **F1 Litters to Day 5 post partum**: At the high-dose level, there was an increase in the number of litters with small female pups [Table 4]. Additionally, it is to be noted that the number of missing and presumed dead pups [both males and females] was increased at the high-dose level compared to the controls. Slightly higher mortality was observed at the high-dose level compared to the control and other dose groups [21%, 21%, 22%, and 30%]. The number of pups per sex per group on day 5 were 85 males/83 females from 21 control litters, 96 males/96 females from 24 low-dose litters, 93 males/90 females from 23 mid-dose litters, and 79 males/80 females from 20 litters. **Selected F1 Pups [days 6 to 29]**: Increased mortality [Table 4] was observed at the high-dose level compared to the control and other dose groups [5, 1, 3, and 24 deaths at the control, low-, mid-, and high-dose levels, respectively]. One mid-dose male [day 7], one mid-dose female [day 7], one high-dose male [day 9], and three high-dose females [days 7-10] were found dead. As was observed above, a higher number of high-dose pups were missing and presumed dead compared to the controls [males 3 vs 9/females 1 vs 8]. There was an increased number of small pups of both sexes at the high-dose level compared to the control.

Table 4. Clinical Observations - F1 Pups				
Observation/Dose	0 ppm	20 ppm	75 ppm	300 ppm
F1 litters [days 1-5]				
# litters w/ small female pups	4	8	7	11
# small female pups	6	10	11	18
# pups missing; presumed dead				
males♪	19/6	27/12	19/10	53/17
females♪	24/9	35/15	34/15	51/20
found dead - # pups (# litters)				
males	9(4)	4(1)	17(7)	8(6)
females	13(5)	7(3)	15(5)	4(4)
found dead/canib☎ - # pups (# litters)				
males	3(1)	0	0	0
females	5(1)	0	0	0
killed in extremis - # pups (# litters)				
males	0	5(1)	0	0
females	0	5(2)	0	0
Total # pups dying/total # born [%]	**73/356** [21]	**83/387** [21]	**85/391** [22]	**116/391** [30]
selected F1 pups [days 6-29]				
small pups				
males	2	0	1	7✘
females	0	0	1	7✘
# pups missing; presumed dead				
males	3	1	0	9✘
females	1	0	1	8✘
found dead				
males	0	0	1	1
females	0	0	1	3✘
killed in extremis				
males	1	0	0	1
females	0	0	0	2
chromodacryorrhea				
males	1	0	0	4
females	1	0	0	6
lachrymation				
males	1	0	0	3
females	0	0	0	3

♪ # pups/# litters w/; ☎ cannibalized; ✘ # litters could not be determined; data from Table 10 [pages 69-70] & Table 12 [pages 74-78] of report

2. Pup Weight and Weight Gain - **F1 Pups** Day 1 post partum mean pup body weight was significantly decreased at the high-dose level compared to the control [91% of the control] for male and female pups combined, although no statistical analyses of the body-weight data for day 1 were provided for the sexes separately for all pups born [Table 5]. All other pup data provided were for the selected F1 pups that continued on test.

Table 5. Body-Weight Data for F1 Pups on Day 1 *post partum*				
Sex/Dose	0 ppm	20 ppm	75 ppm	300 ppm
n=	185	193	194	190
MALES	5.96±0.62	5.78±0.60	5.73±0.54	5.41±0.52
n=	166	191	189	193
FEMALES	5.52±0.58	5.40±0.52	5.33±0.52	5.00±0.50
COMBINED SEXES	5.8±0.6	5.6±0.6	5.5±0.5	5.2±0.5**

Report discussed only selected F1 pups; data in this table are for all F1 pups born; ** $p<0.01$
statistics provided for combined sexes only; data from Table 9 [page 67] and Appendix 8 [pages 945-968] of the report

Selected F1 Pups Decreased body weights were observed at the high-dose level for both sexes from day 5-29 of lactation, and the decrease continued post weaning [days 29-63], although the magnitude of the difference from control in both sexes decreased with time [Table 6]. Females at the mid-dose level displayed a decrease in body weight compared to the control at the end of lactation [days 22-29], with the decrease attaining statistical significance on day 22. Decreased body-weight gains [not provided in the report] were observed in both sexes at the high-dose level, but the decreases occurred mainly during the pre-weaning period [days 5-29].

Table 6. Body Weight and Body-Weight Gain [grams] - Selected F1 Pups

Sex/Day/Dose	0 ppm	20 ppm	75 ppm	300 ppm
BODY WEIGHT				
Females				
5	7.8	7.8	7.6	6.4** [82]
12	18.9	19.2	18.3	13.6** [72]
18	32.8	33.0	31.5	25.4** [77]
22	43.4	43.4	40.7* [94]♦	32.5** [75]
29	76.6	77.4	72.9 [95]	60.3** [79]
36	115.6	116.8	111.6	96.6** [84]
43	148.3	149.9	143.4	129.2** [87]
50	173.2	176.2	168.6	153.9** [89]
57	195.7	197.1	190.3	176.2** [90]
63	206.3	208.2	199.4* [97]	188.0** [91]
BODY WEIGHT				
Males				
5	8.3	8.1	8.2	7.0** [84]
12	19.8	20.0	19.5	14.4** [73]
18	34.4	34.2	33.3	26.6** [77]
22	45.5	44.9	43.4 [95]	34.4** [76]
29	82.8	82.6	79.7 [96]	64.0** [77]
36	130.9	130.8	127.0	106.3** [81]
43	183.9	183.6	178.8	153.4** [83]
50	235.7	235.5	230.4	199.9** [85]
57	287.4	285.7	280.9	248.0** [86]
63	319.7	317.8	312.5	280.2** [88]
BODY-WEIGHT GAIN●				
Females				
days 5-12	11.1	11.4	10.7 [96]	7.2 [65]
days 12-18	13.9	13.8	13.2	11.8 [85]
days 5-22	35.6	35.6	33.1	26.1 [73]
days 5-29	68.8	69.6	65.3	53.9 [78]
days 22-29	33.2	34.0	32.2	27.8 [84]
days 5-63	198.5	200.4	191.8	181.6 [91]
days 36-63	90.7	91.4	87.8	91.4
days 29-63	129.7	130.8	126.5	127.7 [98]

Table 6. Body Weight and Body-Weight Gain [grams] - Selected F1 Pups				
Sex/Day/Dose	0 ppm	20 ppm	75 ppm	300 ppm
BODY-WEIGHT GAIN●				
Males				
days 5-12	11.5	11.9	11.3	7.4 [64]
days 12-18	14.6	14.2	13.8 [95]	12.2 [84]
days 5-22	37.2	36.8	35.2 [95]	27.4 [74]
days 5-29	74.5	74.5	71.5 [96]	57.0 [77]
days 22-29	37.3	37.7	36.3	29.6 [79]
days 5-63	311.4	309.7	304.3	273.2 [88]
days 36-63	188.8	187.0	185.5	173.9 [92]
days 29-63	236.9	235.2	232.8	216.2 [91]

♦ [% of control]; * $p<0.05$; ** $p<0.01$; ● calculated by reviewer using data from Table 14
Data from Table 14, pages 80-81 of the report

As presented, the data do not allow for an assessment of litter survival (presence of at least one viable pup at weaning [Lactation Day 29]), and from the body-weight data provided in Table 14 of the report, the number of males and females weighed on the various days cannot be determined. The number of litters from which F1 pups were selected for continuation on the study is shown in that table, and it is to be noted that 2 of the high-dose litters apparently died between day 5 and day 12 *post partum.*

3. Developmental Landmark Data - There was a significant delay in both preputial separation and vaginal opening at the high-dose level compared to the control groups [Table 7]. These findings may have been correlated to decreased body size, but the method of statistical analysis used did not address this consideration.

Table 7. Developmental Landmarks of Selected F1 Pups - Age [days]				
Parameter/Dose	0 ppm	20 ppm	75 ppm	300 ppm
preputial separation - males	45.2±1.5 [71]♪	45.2±1.3 [83]	45.7±1.6 [80]	47.4±2.2** [60]
vaginal opening – females	35.4±1.8 [71]	35.2±1.3 [84]	35.4±1.4 [77]	36.4±2.0** [58]

♪ [n]; ** $p<0.01$; data from Table 13 [page 79] of the report

4. Mean Auditory Startle Data - On post partum day 23, there was a statistically significant decrease in the startle amplitude [V] for males and females at the 300 ppm dose level at all five intervals [Table 8]. Additionally, the females at this time point displayed a dose-related decrease in the startle amplitude, which was statistically significant at all dose levels in 3 of the 5 intervals. Males displayed comparable responses among the groups on day 61. The high-dose females continued to display a decrease in response at the high-dose level on day 61. Time to maximum amplitude was increased on day 23 in the high-dose males only and only during the second interval. On day 61, the high-dose females displayed a significant increase in the time to maximum amplitude during 4 of the 5 intervals.

Table 8. Auditory Startle Test - Selected F1 Pups

Sex/Day/Dose	0 ppm	20 ppm	75 ppm	300 ppm
STARTLE AMPLITUDE V				
females n=				
day 23	21	24	23	17
repetition 1-10	274.8±128.7	213.3±66.0* [78]♪✖	207.1±83.0* [75]	132.4±37.2** [48]
repetition 11-20	224.9±129.3	169.0±66.9* [75]	151.0±70.9** [67]	99.1±56.5** [44]
repetition 21-30	176.4±78.1	145.7±68.6 [83]	133.7±55.4* [76]	88.2±60.4** [50]
repetition 31-40	160.5±72.8	117.6±63.4* [73]	118.0±50.5* [74]	69.9±53.4** [44]
repetition 41-50	151.4±70.8	131.4±60.5 [87]	116.6±56.1 [77]	64.9±55.6** [43]
day 61				
repetition 1-10	965.5±402.0	995.3±327.5	955.7±283.6	765.2±260.6 [79]
repetition 11-20	876.3±379.3	870.3±301.6	776.0±228.4 [89]	646.7±220.7* [74]
repetition 21-30	708.1±281.3	751.8±219.5	673.9±247.5	488.0±191.5** [69]
repetition 31-40	667.2±271.1	692.0±279.8	649.6±259.5	527.4±261.8 [79]
repetition 41-50	649.2±332.6	613.9±236.8	591.9±256.0 [91]	508.9±222.7 [78]
STARTLE AMPLITUDE V				
Males n=				
day 23	21	24	23	16
repetition 1-10	248.9±90.9	214.3±61.8	255.4±142.8	162.0±57.8** [65]
repetition 11-20	198.8±81.4	178.1±62.3	192.1±77.0	124.7±61.7** [63]
repetition 21-30	160.6±71.3	148.9±50.9	160.1±77.8	103.2±54.5** [64]
repetition 31-40	165.7±89.7	130.8±51.4	136.0±61.6	107.3±53.2** [65]
repetition 41-50	157.9±96.4	125.6±60.2	132.5±59.8	102.2±53.1* [65]
day 61				
repetition 1-10	1299.4±372.7	1207.0±356.5	1347.8±407.1	1266.4±268.7
repetition 11-20	959.5±328.7	983.4±431.9	1051.7±364.5	1110.9±245.6
repetition 21-30	942.0±302.3	944.1±371.7	1022.7±476.2	976.4±329.5
repetition 31-40	860.6±406.1	853.5±336.8	768.2±366.0	909.3±277.2
repetition 41-50	851.3±381.9	886.6±407.9	791.5±313.5	841.6±262.7
TIME TO MAXIMUM AMP [MS]				
Females				
day 23				
repetition 1-10	28.2±8.8	28.0±6.1	27.0±4.6	28.5±9.2
repetition 11-20	22.5±5.5	20.1±1.9	21.7±3.8	23.1±5.3
repetition 21-30	21.3±3.2	20.0±2.6	20.2±3.0	22.1±4.0
repetition 31-40	21.4±5.1	19.7±3.2	20.4±3.7	19.7±7.7 [92]
repetition 41-50	20.9±3.2	19.5±3.8	19.7±3.6	18.3±7.7 [88]
day 61				
repetition 1-10	25.5±5.2	25.6±4.8	23.9±2.6	28.0±4.2± [110]
repetition 11-20	23.1±5.8	23.3±4.0	23.2±4.1	26.9±3.4* [116]
repetition 21-30	23.7±2.9	22.8±2.4	22.5±2.6	27.0±4.3** [114]
repetition 31-40	22.2±3.0	23.6±3.2	23.5±3.4	25.3±3.4** [114]
repetition 41-50	23.0±3.4	23.5±3.1	23.7±3.4	25.7±3.8* [112]

Table 8. Auditory Startle Test - Selected F1 Pups				
Sex/Day/Dose	0 ppm	20 ppm	75 ppm	300 ppm
TIME TO MAXIMUM AMP [MS]				
Males				
day 23				
repetition 1-10	26.5±7.7	24.2±3.7	27.6±4.8	30.2±7.5 [114]
repetition 11-20	21.7±3.1	20.9±2.2	21.3±3.3	24.1±4.2* [111]
repetition 21-30	21.0±2.7	20.4±3.1	22.0±4.2	20.6±4.3
repetition 31-40	21.3±3.9	20.5±3.2	21.5±3.2	23.9±7.5 [112]
repetition 41-50	22.2±5.4	20.2±2.9	20.3±2.2	20.6±1.8
day 61				
repetition 1-10	26.2±5.7	25.2±4.2	27.2±6.1	25.8±2.1
repetition 11-20	22.8±2.0	24.4±4.0	23.4±2.8	23.8±3.6
repetition 21-30	23.3±2.4	23.8±3.0	24.5±3.5	23.1±2.3
repetition 31-40	23.6±3.0	23.4±1.8	25.0±3.4	22.8±1.6
repetition 41-50	23.7±2.4	24.4±3.2	24.0±2.6	23.4±2.1

♪ [% of control]; * p<0.05; ** p<0.01
Data from Table 17, pages 99-106 of the report
✖ calculated by reviewer using data from Table 17

7. Mean Motor Activity Data – **MALES** On day 14 *post partum*, the high-dose males consistently displayed a lower motor activity level than the control males [Table 9]. Slightly elevated activity levels were displayed at all time points on day 18 *post partum* by this group, and there was a statistically significant increase in mean motor activity counts on day 22 *post partum* for this group of males. On day 60 *post partum*, the high-dose males continued to displayed a slight increase in activity level over the control value. Because of the initial decrease and subsequent sustained increase in motor activity at the high-dose level compared to the control, an effect on this parameter cannot be ruled out for males at the 300 ppm dose level. **FEMALES** Comparable levels of motor activity were observed among the groups of females throughout the study.

Table 9. Motor Activity [units not provided]				
Day/Interval/minutes/Sex/Dose	0 ppm	20 ppm	75 ppm	300 ppm
day 14				
MALES				
1-5	18.6±27.6	13.2±21.1	18.3±20.5	10.3±16.2
6-10	10.0±12.5	8.3±13.3	11.0±16.0	3.0±5.7
11-15	6.1±9.5	3.4±6.0	5.5±10.0	2.2±5.6
16-20	4.7±10.7	3.4±5.7	2.3±6.0	0.8±2.8
21-25	3.4±6.3	1.8±4.1	0.8±1.6	2.1±5.0
26-30	5.1±9.2	3.8±9.4	4.0±10.3	1.0±3.1
31-35	2.3±5.1	0.8±1.9	2.9±6.8	0.2±0.7
36-40	1.4±2.7	1.5±3.7	0.3±0.9	0.4±1.7
41-45	1.0±2.6	0.7±1.5	1.2±3.2	0.3±0.8
46-50	0.5±1.1	0.4±1.0	2.1±6.6	0.3±0.8
overall	53.1±56.2	37.2±50.0	48.5±60.2	20.7±32.2

Table 9. Motor Activity [units not provided]				
Day/Interval/minutes/Sex/Dose	0 ppm	20 ppm	75 ppm	300 ppm
day 14				
FEMALES				
1-5	19.4±22.8	33.6±27.6	25.4±28.2	26.2±25.7
6-10	14.3±17.0	13.5±13.3	21.3±23.6	16.5±20.5
11-15	14.9±20.8	15.5±19.9	8.1±16.7	10.6±16.8
16-20	7.6±10.1	14.0±15.3	6.8±12.3	7.1±16.4
21-25	3.7±5.9	7.0±9.2	8.0±14.1	7.0±15.3
26-30	6.2±10.6	6.4±10.5	5.9±11.9	5.3±10.3
31-35	7.6±13.0	10.2±16.6	4.2±10.4	2.9±9.9
36-40	1.6±3.7	6.1±10.9	3.2±12.5	2.4±5.6
41-45	2.6±5.7	2.4±5.0	1.3±3.9	2.1±4.0
46-50	2.7±7.1	6.8±14.6	2.1±5.5	1.1±3.2
overall	80.4±90.2	115.5±105.4	86.4±95.9	81.3±110.9
day 18				
MALES				
1-5	19.7±20.7	23.5±24.8	23.0±18.7	22.3±17.2
6-10	20.7±21.4	21.4±17.3	23.8±19.3	22.5±21.9
11-15	17.4±19.6	20.3±17.6	21.8±16.2	24.1±21.0
16-20	16.6±20.2	16.1±14.8	29.3*±21.4	25.7±16.4
21-25	16.0±23.1	15.5±15.0	16.7±13.2	18.4±17.5
26-30	15.0±15.5	14.9±15.1	19.1±19.6	24.6±22.2
31-35	20.1±23.7	15.9±15.2	15.3±19.7	20.7±21.4
36-40	10.8±16.6	11.7±14.4	14.9±18.3	17.0±15.8
41-45	16.4±21.3	10.4±14.9	10.7±16.1	17.3±17.8
46-50	14.7±22.1	14.3±17.9	13.5±18.7	18.2±21.3
overall	167.4±170.4	163.9±99.4	188.2±124.7	210.6±141.2
day 18				
FEMALES				
1-5	31.9±27.1	32.1±22.2	24.7±25.1	39.0±24.5
6-10	29.2±27.3	29.0±18.9	21.8±22.1	34.4±18.5
11-15	25.4±24.5	27.2±23.1	22.9±18.0	30.5±17.6
16-20	25.9±25.1	20.0±21.2	18.0±20.6	24.0±15.4
21-25	31.2±20.5	16.2*±21.9	21.9±20.1	31.2±20.2
26-30	27.5±24.1	15.5±18.6	18.8±21.7	22.8±20.0
31-35	28.1±28.0	21.7±24.0	16.8±20.2	21.1±20.3
36-40	21.8±24.4	13.8±18.3	14.0±19.3	25.0±18.7
41-45	16.4±19.1	16.2±21.7	12.9±17.9	21.6±21.2
46-50	21.5±22.4	7.0*±11.6	13.3±16.4	14.3±23.3
overall	258.8±188.4	198.5±146.7	185.0±155.8	263.9±131.1
Day 22				
MALES				
1-5	25.7±19.1	33.1±22.1	30.0±17.4	35.6±18.4
6-10	29.0±19.6	35.0±16.5	35.8±17.5	38.1±21.9
11-15	32.4±16.6	29.8±16.9	35.8±16.8	39.5±18.3
16-20	30.1±15.9	35.3±21.1	30.0±18.1	41.9*±13.8
21-25	35.0±20.7	26.5±21.2	32.7±19.9	41.4±20.9
26-30	22.7±20.1	25.0±13.9	30.1±19.3	35.9*±18.6
31-35	27.2±18.6	23.6±18.8	20.7±18.0	39.6*±19.4
36-40	31.0±23.1	26.8±22.5	26.2±22.3	34.0±22.7
41-45	25.2±18.1	13.5*±13.6	21.3±19.8	35.2±18.7
46-50	21.4±18.4	15.7±17.9	27.2±20.6	35.2*±25.4
overall	279.8±110.7	264.3±108.6	290.0±125.5	376.4*±126.7

Table 9. Motor Activity [units not provided]				
Day/Interval/minutes/Sex/Dose	0 ppm	20 ppm	75 ppm	300 ppm
day 22				
FEMALES				
1-5	39.9±21.2	42.8±19.9	38.2±18.5	39.9±16.3
6-10	39.2±19.3	45.2±18.5	36.2±19.1	41.3±14.6
11-15	38.1±19.8	41.4±21.5	38.0±16.9	43.2±22.3
16-20	41.7±16.5	36.4±22.4	39.3±20.6	39.3±19.0
21-25	38.7±19.1	37.9±24.0	38.4±18.3	39.4±21.6
26-30	40.7±20.1	29.1±19.1	32.9±21.4	38.5±20.1
31-35	33.8±20.6	27.0±21.9	31.7±17.7	37.7±23.2
36-40	39.6±20.9	22.8**±21.3	33.9±19.7	38.6±20.2
41-45	34.4±26.8	23.7±25.1	26.8±21.1	38.5±17.2
46-50	31.6±21.9	23.6±18.5	23.5±22.9	31.6±23.2
overall	377.8±159.3	329.8±124.9	338.9±143.3	388.1±153.3
day 60				
MALES				
1-5	70.0±13.5	70.3±11.7	70.3±12.3	69.9±13.2
6-10	66.3±12.3	66.5±12.7	69.3±14.4	68.4±13.4
11-15	63.6±11.3	63.6±13.5	68.7±11.6	66.5±10.7
16-20	63.4±9.3	62.9±11.1	62.5±13.1	63.4±10.4
21-25	62.7±10.9	62.2±9.6	61.0±18.7	67.2±5.6
26-30	60.0±13.9	61.0±15.5	59.5±23.0	62.1±20.4
31-35	52.9±16.6	49.5±20.9	56.8±20.1	64.3±19.4
36-40	48.0±23.0	44.1±29.9	42.4±24.7	54.1±15.1
41-45	36.4±31.4	33.8±30.4	37.2±29.0	51.8±22.6
46-50	25.4±29.1	22.8±27.4	25.0±27.1	37.3±26.9
overall	548.7±122.0	536.9±131.9	552.9±129.3	605.0±115.1
day 60				
FEMALES				
1-5	66.3±11.7	60.7±13.4	57.3*±13.2	57.8*±9.9
6-10	61.8±10.7	59.0±11.8	54.8±14.3	58.2±11.6
11-15	61.9±10.4	62.0±13.1	59.0±15.7	56.1±11.5
16-20	63.1±11.1	62.0±11.4	60.8±14.1	60.8±15.7
21-25	62.1±11.1	63.0±8.1	55.0±14.2	62.3±15.8
26-30	60.6±12.7	61.1±11.0	57.1±16.7	59.5±12.0
31-35	61.4±12.5	61.2±15.1	52.6*±14.1	60.1±12.6
36-40	59.1±15.5	61.1±18.2	53.0±20.3	54.6±18.4
41-45	55.7±23.7	56.7±20.6	48.8±23.1	55.5±18.4
46-50	51.9±23.6	50.6±25.4	48.4±22.1	54.8±19.1
overall	603.9±102.3	597.3±94.1	547.0±138.0	579.6±101.2

* $p<0.05$; ** $p<0.01$; data from Table 15 [pages 82-89] of the report

6. Learning and memory tests – **STRAIGHT CHANNEL:** Swimming time was increased [Table 10] on day 21 *post partum* at the high-dose level for both sexes compared to the controls but comparable among the groups for both sexes at the other three time points. **Y-SHAPED WATER MAZE: Learning phase [day 21]** At the high-dose level, both sexes had a lower percentage of successful trials compared to the controls throughout the test [Table 11]. **Memory phase [day 24]** Both sexes at the high-dose level displayed a lower percentage of successful trials throughout the test. **Learning phase [day 59]** Males displayed comparable successes among the groups, and the fewer successful trials noted for the females at the mid- and low-dose levels showed no dose response. **Memory phase [day 62]** No apparent differences were observed among the groups for either sex.

Table 10. STRAIGHT CHANNEL SWIM [Learning and Memory Tests]				
Sex/Dose/Time [phase]	0 ppm	20 ppm	75 ppm	300 ppm
MALES				
Straight channel				
Day 21 [learning]	6.33±5.98	5.95±4.46	5.51±3.47	7.94±8.58
Day 24 [memory]	3.16±1.29	3.23±1.17	3.22±1.38	2.90±0.68
Day 59 [learning]	2.44±0.36	2.43±0.37	2.32±0.40	2.33±0.32
Day 62 [memory]	2.07±0.29	2.17±0.46	2.18±0.52	2.13±0.29
FEMALES				
Straight channel				
Day 21 [learning]	4.62±3.07	4.25±2.40	4.49±1.96	8.30**±7.06
Day 24 [memory]	2.90±0.91	2.85±0.99	3.02±1.23	3.47±1.60
Day 59 [learning]	2.72±0.95	2.40±0.52	2.64±0.61	2.40±0.47
Day 62 [memory]	1.98±0.18	2.05±0.27	2.09±0.27	2.15±0.36

** $p<0.01$; Data from Table 16, pages 90-98 of the report

Table 11. Learning and Memory Tests [% of successful trials]				
Phase/Sex/Dose	0 ppm	20 ppm	75 ppm	300 ppm
MALES				
Day 21 learning phase				
cut-off 3 sec	1.6±5.0	0.7±3.4	2.9±10.8	0.0±0.0
cut-off 4 sec	11.1±15.2	11.1±19.5	10.9±17.1	2.8±8.6
cut-off 5 sec	19.8±24.5	22.2±20.1	19.6±19.9	7.4±16.4
cut-off 6 sec	30.2±27.7	33.3±21.4	27.5±24.4	13.9*±20.0
cut-off 7 sec	37.3±29.3	38.9±21.2	35.5±26.7	22.2±23.6
cut-off 8 sec	44.4±31.3	49.3±20.0	42.0±27.0	29.6±24.6
cut-off 9 sec	50.0±29.3	54.2±21.0	48.6±28.4	34.3±27.7
cut-off 10 sec	56.3±32.7	60.4±21.3	56.5±32.1	43.5±26.3
FEMALES				
Day 21 learning phase				
cut-off 3 sec	3.2±10.0	0.7±3.4	0.8±3.6	1.9±7.9
cut-off 4 sec	14.3±19.9	17.4±18.7	9.8±14.2	5.6±11.4
cut-off 5 sec	23.0±26.6	27.8±19.5	22.0±19.5	15.7±20.2
cut-off 6 sec	30.2±26.2	36.8±23.0	31.1±23.2	20.4±21.8
cut-off 7 sec	35.7±27.0	45.1±23.3	38.6±23.8	26.9±20.7
cut-off 8 sec	40.5±29.1	54.9*±22.2	43.9±23.3	35.2±18.9
cut-off 9 sec	46.8±27.2	59.7±20.2	47.0±22.2	38.9±19.8
cut-off 10 sec	55.6±26.0	64.6±18.6	51.5±21.2	41.7*±19.2
MALES				
Day 24 memory phase				
cut-off 3 sec	29.4±25.8	29.9±20.3	31.2±29.4	13.0*±22.5
cut-off 4 sec	50.8±26.1	59.7±18.3	53.6±26.1	50.0±27.4
cut-off 5 sec	64.3±22.5	63.9±17.5	66.7±25.6	59.3±26.9
cut-off 6 sec	70.6±22.3	73.6±16.2	74.6±24.0	63.9±24.4
cut-off 7 sec	79.4±22.3	81.3±14.2	80.4±21.7	69.4±25.1
cut-off 8 sec	83.3±23.6	87.5±10.1	86.2±14.8	75.0±24.4
cut-off 9 sec	86.5±20.2	91.0±11.0	88.4±12.7	76.9±23.7
cut-off 10 sec	89.7±19.3	94.4±8.0	91.3±11.1	82.4±22.5

Table 11. Learning and Memory Tests [% of successful trials]				
Phase/Sex/Dose	0 ppm	20 ppm	75 ppm	300 ppm
FEMALES				
Day 24 memory phase				
cut-off 3 sec	31.0±29.0	30.6±25.4	37.1±30.0	21.3±26.1
cut-off 4 sec	55.6±23.2	50.0±25.5	59.1±21.0	46.3±34.1
cut-off 5 sec	70.6±17.4	59.7±20.8	65.2±20.5	57.4±29.8
cut-off 6 sec	80.2±17.2	70.8±18.6	68.9*±16.5	64.8*±25.5
cut-off 7 sec	84.1±17.9	77.8±17.5	75.8±15.2	71.3*±27.3
cut-off 8 sec	85.7±17.7	83.3±17.7	82.6±14.1	76.9±26.9
cut-off 9 sec	89.7±14.4	88.9±14.5	87.1±12.5	82.4±20.2
cut-off 10 sec	94.4±8.1	90.3±13.8	90.0±11.2	88.9±14.0
MALES				
Day 59 learning phases				
Cut off 3 sec	26.2±29.1	18.1±22.5	24.6±20.0	19.4±18.3
cut-off 4 sec	42.1±29.2	34.7±21.9	39.1±26.4	37.0±18.6
cut-off 5 sec	56.3±25.0	51.4±21.9	55.1±24.8	47.2±24.4
cut-off 6 sec	70.6±18.9	65.3±21.9	63.8±20.5	68.5±21.3
cut-off 7 sec	76.2±17.1	75.7±17.0	75.4±16.6	75.9±17.4
cut-off 8 sec	81.0±14.2	84.7±15.5	84.1±13.7	80.6±18.3
cut-off 9 sec	86.5±12.5	89.6±13.7	87.0±14.2	82.4±19.4
cut-off 10 sec	88.1±13.1	91.0±13.0	92.0±11.1	85.2±13.9
FEMALES				
Day 59 learning phase				
cut-off 3 sec	26.2±24.5	34.0±20.5	22.7±18.9	22.2±23.6
cut-off 4 sec	46.8±23.3	49.3±21.7	34.8±23.5	37.0±27.1
cut-off 5 sec	58.7±21.5	64.6±21.0	42.4*±22.3	53.7±25.9
cut-off 6 sec	67.5±17.1	69.4±19.5	56.1±23.9	64.8±26.1
cut-off 7 sec	74.6±17.2	72.9±19.5	65.9±23.3	72.2±18.1
cut-off 8 sec	80.2±17.2	76.4±15.5	71.2±23.1	76.9±16.3
cut-off 9 sec	87.3±14.8	78.5±15.9	75.0*±24.0	79.6±16.7
cut-off 10 sec	90.5±15.4	81.3*±15.0	78.8*±20.7	82.4±17.6
MALES				
Day 62 memory phase				
cut-off 3 sec	57.1±36.0	59.7±25.5	54.3±28.1	55.6±30.2
cut-off 4 sec	65.9±33.1	75.0±19.0	62.3±28.1	71.3±20.5
cut-off 5 sec	72.2±32.2	81.3±15.0	70.3±25.1	83.3±19.8
cut-off 6 sec	77.8±29.0	88.9±10.6	78.3±24.3	86.1±20.0
cut-off 7 sec	84.9±24.7	93.1±9.7	84.1±18.4	88.9±19.0
cut-off 8 sec	87.3±21.7	95.8±8.9	86.2±18.6	91.7±15.4
cut-off 9 sec	93.7±16.2	95.8±8.9	89.9±16.5	94.4±11.4
cut-off 10 sec	96.0±10.4	96.5±8.5	92.0±13.2	96.3±9.1
FEMALES				
Day 62 memory phase				
cut-off 3 sec	65.1±20.3	66.7±27.8	61.4±30.6	63.0±25.9
cut-off 4 sec	75.4±19.5	75.0±22.5	68.2±25.7	73.1±23.7
cut-off 5 sec	77.8±18.5	78.5±18.7	72.7±20.9	81.5±17.0
cut-off 6 sec	81.7±15.7	81.3±17.9	80.3±16.8	85.2±12.6
cut-off 7 sec	91.3±11.3	88.2±13.4	84.8±15.4	89.8±11.6
cut-off 8 sec	92.9±10.0	92.4±11.0	86.4±14.2	93.5±10.1
cut-off 9 sec	92.9±10.0	95.1±9.2	90.2±14.2	94.4±9.9
cut-off 10 sec	94.4±9.6	95.8±8.9	90.0±13.3	96.3±7.1

* $p<0.05$; ** $p<0.01$; data from Table 16, pages 91-98 of the report

C. POSTMORTEM EXAMINATIONS

1. Brain Weight Data (Tables 12 & 13) – **Day 12** Statistically significant decreases in absolute brain weights were observed in both sexes at the high-dose level compared to the controls. Although terminal body weights were decreased [not statistically significant] at the high-dose level, a biologically significant delay in brain development is demonstrated by these data. The relative brain weight for both sexes was not significantly increased in comparison to the controls. **Day 63** Similar statistically significant decreases in absolute brain weights were observed in both sexes at the high-dose level compared to the controls at this time point also, along with a decrease in terminal body weight [not statistically significant] at the high-dose level. Additionally, the relative brain weight for both sexes was not significantly increased in comparison to the controls. The group mean absolute and relative brain weights for the low- and mid-dose groups were comparable to the control groups. The decreased brain weight at 300 ppm is considered treatment-related in both sexes.

Table 12. Mean Male Pup Brain Weights (g)

Molinate Treatment Level (ppm)	0	20	75	300
Day 12				
Terminal Body Weight [n] (grams)	18.1±3.5 [10]	19.5±2.7 [12]	18.7±3.3 [12]	14.9±2.1 [8]
Absolute Brain Weight (grams)	0.98±0.11	1.01±0.08	0.97±0.09	0.87*±0.06
Relative Brain Weight [%]	5.56±0.91	5.23±0.53	5.27±0.64	5.89±0.57
Brain Weight Adjusted for Body Weight (grams)	0.98	0.98	0.95	0.94
Day 63				
Terminal Body Weight [n] (grams)	323.6±23.7 [19]	316.7±19.5 [20]	307.7±26.6 [19]	282.3±22.2 [17]
Absolute Brain Weight (grams)	1.84±0.13	1.82±0.10	1.78±0.13	1.72**±0.11
Relative Brain Weight [%]	0.57±0.04	0.58±0.04	0.58±0.05	0.61±0.05
Brain Weight Adjusted for Body Weight (grams)	1.81	1.80	1.78	1.77

Data from Table 18 (pages 107 & 110) of the study report. * $p<0.05$; ** $p<0.01$

Table 13. Mean Female Pup Brain Weights (g)

Molinate Treatment Level (ppm)	0	20	75	300
Day 12				
Terminal Body Weight [n] (grams)	18.6±2.9 [11]	19.9±2.3 [12]	19.3±3.8 [11]	13.6±2.7 [9]
Absolute Brain Weight (grams)	0.96±0.09	0.99±0.09	0.97±0.09	0.82**±0.12
Relative Brain Weight [%]	5.25±0.66	5.01±0.44	5.18±0.74	6.09±0.66
Brain Weight Adjusted for Body Weight (grams)	0.95	0.95	0.95	0.93
Day 63				
Terminal Body Weight [n] (grams)	202.4±12.5 [18]	206.9±15.7 [20]	197.2±14.5 [20]	183.7±14.3 [17]
Absolute Brain Weight (grams)	1.70±0.10	1.71±0.10	1.71±0.09	1.59**±0.13
Relative Brain Weight [%]	0.84±0.06	0.83±0.07	0.87±0.06	0.87±0.08
Brain Weight Adjusted for Body Weight (grams)	1.69	1.69	1.71	1.62*

Data from Table 18 (pages 107 & 110) of the study report. * $p<0.05$; ** <0.01

2. Brain Length and Width Data – **Day 12** Significantly decreased brain length was observed in both sexes at the high dose, and significantly decreased brain width was observed in the high-dose females [Tables 14 & 15]. **Day 63** Slight decreases in both the length and width were observed at the high dose [both sexes], but

a $p<0.05$ was not attained.

Table 14: Mean Male Pup Brain Lengths and Widths (mm)[a]				
Molinate Treatment Level (ppm)	0	20	75	300
Day 12				
Brain Length	18.9±1.2	19.0±1.2	18.9±0.8	17.6*±0.9
Relative Brain Length [%]	108.0±20.5	98.5±10.6	104.0±17.8	120.2±16.3
Brain Length Adjusted to Body Weight	18.9	18.7	18.8	18.2
Brain Width	12.6±0.5	12.8±0.6	12.6±0.5	12.1±0.6
Relative Brain Width [%]	72.1±14.0	66.3±8.3	69.3±12.1	82.6±10.6
Brain Width Adjusted to Body Weight	12.6	12.6	12.5	12.4
Day 63				
Brain Length	25.9±0.8	26.0±1.1	26.1±1.0	25.5±1.1
Relative Brain Length [%]	8.1±0.7	8.2±0.7	8.5±0.8	9.1±1.0
Brain Length Adjusted to Body Weight	26.0	26.0	26.1	25.4
Brain Width	14.7±0.5	14.8±0.5	14.7±0.5	14.5±0.5
Relative Brain Width [%]	4.6±0.3	4.7±0.2	4.8±0.4	5.2±0.4
Brain Width Adjusted to Body Weight	14.6	14.7	14.7	14.8

a Data from Table 18 (pages 108, 109, 111, 112) of the study report.; * $p<0.05$; ** $p<0.01$

Table 15. Mean Female Pup Brain Lengths and Widths (mm)[a]				
Molinate Treatment Level (ppm)	0	20	75	300
Day 12				
Brain Length	18.5±0.7	18.9±0.5	18.7±0.9	17.2**±1.4
Relative Brain Length [%]	102.0±17.5	96.3±9.6	100.7±19.5	129.4±18.0
Brain Length Adjusted to Body Weight	18.4	18.6	18.5	18.1
Brain Width	12.5±0.5	12.8±0.5	12.6±0.5	11.8**±0.7
Relative Brain Width [%]	68.5±11.8	64.9±6.3	68.1±14.0	88.9±15.1
Brain Width Adjusted to Body Weight	12.4	12.6	12.5	12.2
Day 63				
Brain Length	25.2±1.1	25.5±1.2	25.9±1.1	24.8±1.0
Relative Brain Length [%]	12.5±1.0	12.4±1.2	13.2±1.1	13.6±1.2
Brain Length Adjusted to Body Weight	25.2	25.5	25.8	24.8
Brain Width	14.7±0.6	14.6±0.5	14.7±0.5	14.5±0.5
Relative Brain Width [%]	7.3±0.5	7.1±0.5	7.5±0.5	7.9±0.7
Brain Width Adjusted to Body Weight	14.7	14.5	14.7	14.5

a Data from Table 18 (pages 108, 109, 111, 112) of the study report. * $p<0.05$; ** $p<0.01$

3. Gross Postmortem Examination of Pups - There was no evidence of a treatment-related effect on the gross postmortem findings for pups sacrificed on Days 12 and 63.

4. Histopathological Examinations - There were no microscopic morphologic abnormalities in the brains of any of the pups sacrificed on day 12, and there were no changes in the central or peripheral nervous systems on day 63 that could be attributed to treatment.

5. Morphometric Measurements – **Day 12** Treatment-related changes in the cortex and/or cerebellum of the brain were observed at the mid- and high-dose levels [Tables 16 and 17]. In the high-dose females, there was a slight decrease in frontal cortex height ($p\leq0.05$) [2A] and width [2B]. In both sexes at the high-dose level, slight decreases in the thickness of the lateral cortex [3B] and piriform cortex [4B] were observed. There were also slight decreases in cerebellar height [8H] and length [8L] and in the thickness of the inner granular [8PCFI] and molecular [8PCFM] layers ($p\leq 0.05$ for femlaes) of the prepyramidal fissure at the high-dose level. Slight decreases in cerebellar height [8H], thickness of the inner granular [8PCFI] layer of the

preculminate fissure and/or thickness of the inner granular [8PPFI] and molecular [8PPFM] layers of the prepyramidal fissure were observed at the mid-dose level. **Day 63** – Treatment-related changes in the cortex, hippocampus and/or cerebellum were observed at the high-dose level [Tables 18 and 19]. There was a slight decrease in the frontal cortex height [2A] and width [2B], and a slight decrease in the length of the hippocampus from the midline [3D and 4G] in both sexes at the high-dose level. Additionally, there was a slight decrease in cerebellar length [8L] and a slight increase in the thickness of the inner granular [8PCFI] and molecular [8PCFM] layers of the preculminate fissure at the high-dose level. A slight reduction in the thickness of the piriform cortex was observed at the 3C level for rats from all treatment groups, but there was no clear dose response.

Day/Dose [ppm]	Table 16. Morphometric Findings in Male Pup Brains[a]			
	0.0000	20	75	300
Day 12				
Terminal Body Weight [n]	17.2±3.6 [6]	18.8±3.2 [6]	18.3±3.6 [6]	15.8±1.8 [5]
Frontal cortex height [mm]	4.986±0.255	4.759±0.173	4.801±0.290	4.863±0.504
Frontal cortex width [mm]	3.876±0.367	3.585±0.163	3.856±0.136	3.598±0.356
Hippocampus length from midline [mm]	1.790±0.148	1.986±0.127	1.884±0.202	1.799±0.394
Lateral cortex thickness [mm]	0.948±0.117	0.974±0.077	1.024±0.050	0.914±0.027
Piriform cortex thickness [mm]	0.754±0.067	0.737±0.097	0.714±0.044	0.650*±0.052
Corpus callosum thickness [mm]	0.331±0.051	0.341±0.005	0.356±0.026	0.361±0.050
Thalamus/cortex overall width [mm]	9.742±0.933	9.642±0.708	9.728±0.667	9.622±0.646
Hippocampus length from midline [mm]	2.859±0.369	3.105±0.255	2.717±0.178	3.001±0.357
Hippocampus length dentate gyrus [mm]	0.998±0.131	0.976±0.128	1.029±0.112	1.055±0.170
Cerebellum height [mm]	3.098±0.348	3.016±0.399	2.851±0.332	2.807±0.528
Cerebellum length [mm]	3.795±0.393	3.458±0.457	3.676±0.333	3.462±0.629
Cerebellum preculminate fissure thickness of inner granular layer [µm]	105.981±23.041	122.583±21.871	117.027±18.261	92.818±21.078
Cerebellum preculminate fissure thickness of molecular layer [µm]	50.125±11.114	53.511±7.560	54.006±10.034	46.808±10.375
Cerebellum prepyramidal fissure thickness of inner granular layer [µm]	115.471±31.171	123.095±28.326	106.775±12.802	87.091±8.716
Cerebellum prepyramidal fissure thickness of molecular layer [µm]	53.578±16.270	50.905±8.768	47.806±12.925	36.601±8.052
Cerebellum prepyramidal fissure thickness of outer granular layer [µm]	35.562±8.388	39.231±14.450	32.841±7.013	36.844±9.805

a Data from Table 21 (pages 126-153) of the study report.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

Day/Dose [ppm]	Table 17. Morphometric Findings in Female Pup Brains[a]			
	0.0000	20	75	300
Day 12				
Terminal Body Weight [n]	18.7±3.0 [6]	19.6±1.5 [6]	19.3±3.0 [6]	13.6±3.4 [6]
Frontal cortex height [mm]	5.076±0.359	4.769±0.364	4.703±0.439	4.422*±0.456
Frontal cortex width [mm]	3.708±0.201	3.623±0.417	3.766±0.395	3.499±0.413
Hippocampus length from midline [mm]	1.894±0.430	1.878±0.198	1.693±0.249	1.749±0.188
Lateral cortex thickness [mm]]	0.985±0.055	0.961±0.079	1.026±0.081	0.931±0.060
Piriform cortex thickness [mm]	0.759±0.146	0.712±0.112	0.734±0.054	0.686±0.094
Corpus callosum thickness [mm]	0.308±0.100	0.327±0.057	0.378±0.039	0.339±0.009
Thalamus/cortex overall width [mm]	9.527±0.347 [5]	9.411±0.721 [4]	9.435±0.238 [3]	9.311±0.588 [5]
Hippocampus length from midline [mm]	2.786±0.266	2.738±0.325	2.823±0.127	2.694±0.306
Hippocampus length dentate gyrus [mm]	1.036±0.143	1.015±0.138	0.990±0.097	1.013±0.155
Cerebellum height [mm]	2.922±0.300	2.855±0.252	2.730±0.336	2.747±0.295 [2]
Cerebellum length [mm]	3.556±0.624	3.556±0.205	3.499±0.571	3.359±0.236 [2]
Cerebellum preculminate fissure thickness of inner granular layer [µm]	139.631±22.017	113.353±25.568	116.766±12.484	114.853±24.075
Cerebellum preculminate fissure thickness of molecular layer [µm]	61.739±11.626	61.727±16.865	58.687±6.965	46.580±12.141
Cerebellum prepyramidal fissure thickness of inner granular layer [µm]	115.785±19.431	117.785±14.110	96.880±21.229	83.565±24.525
Cerebellum prepyramidal fissure thickness of molecular layer [µm]	62.057±18.216	62.099±13.587	42.182*±7.865	28.997**±2.273 [2]
Cerebellum prepyramidal fissure thickness of outer granular layer [µm]	33.213±7.692	33.244±10.265	32.845±7.137	38.915±9.840 [2]

a Data from Table 21 (pages 126-153) of the study report.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

Day/Dose [ppm]	Table 18. Morphometric Findings in Male Pup Brains[a]			
	0	20	75	300
Day 63				
Terminal Body Weight [n]	314.4±19.8 [7]	311.0±24.4 [6]	312.0±18.0 [6]	288.8±16.5 [8]
Frontal cortex height [mm]	5.348±0.347	5.327±0.436	5.111±0.432	4.958±0.282
Frontal cortex width [mm]	4.148±0.181	4.289±0.385	4.014±0.613	3.948±0.413
Hippocampus length from midline [mm]	1.887±0.506	2.084±0.350	2.087±0.704	1.421±0.293
Piriform cortex thickness [mm]	0.914±0.102	0.831±0.072	0.852±0.051	0.908±0.068
Corpus callosum thickness [mm]	0.374±0.058	0.315±0.101	0.357±0.022	0.344±0.095
Thalamus/cortex overall width [mm]	12.332±0.406	12.393±0.403	11.885±0.337	11.954±0.401
Hippocampus length from midline [mm]	2.952±0.522	3.280±0.383	3.255±0.450	2.410*±0.417
Hippocampus length dentate gyrus [mm]	1.144±0.154	1.248±0.249	1.240±0.251	0.989±0.095
Cerebellum height [mm]	4.422±0.301	4.429±0.410	4.668±0.322	4.531±0.270
Cerebellum length [mm]	6.034±0.286	6.112±0.391	5.896±0.196	5.915±0.324

Day/Dose [ppm]	Table 18. Morphometric Findings in Male Pup Brains[a]			
	0	20	75	300
Day 63				
Cerebellum preculminate fissure thickness of inner granular layer [µm]	131.536±25.715	122.937±25.676	135.064±36.204	140.164±26.357
Cerebellum preculminate fissure thickness of molecular layer [µm]	163.970±30.809	172.803±26.833	178.155±31.852	174.529±35.475
Cerebellum prepyramidal fissure thickness of inner granular layer [µm]	124.494±13.783	112.961±18.485	124.713±12.773	123.520±10.480
Cerebellum prepyramidal fissure thickness of molecular layer [µm]	164.692±14.949	152.350±13.567	174.441±17.653	160.836±9.150

a Data from Table 21 (pages 154-179) of the study report.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

Day/Dose [ppm]	Table 19. Morphometric Findings in Female Pup Brains[a]			
	0	20	75	300
Day 63				
Terminal Body Weight [n]	203.3±9.5 [7]	217.2±23.8 [5]	198.1±14.5 [8]	182.5±19.4 [8]
Frontal cortex height [mm]	4.954±0.274	4.933±0.158	5.152±0.353	4.902±0.391
Frontal cortex width [mm]	3.986±0.306	4.135±0.299	4.238±0.270	3.795±0.451
Hippocampus length from midline [mm]	1.696±0.534	1.984±0.725	2.037±0.548	1.697±0.361
Piriform cortex thickness [mm]	0.893±0.143	0.853±0.026	0.891±0.086	0.821±0.063
Corpus callosum thickness [mm]	0.342±0.092	0.358±0.083	0.344±0.086	0.339±0.074
Thalamus/cortex overall width [mm]	11.703±0.410	12.223±0.643	11.731±0.183	11.292±0.608
Hippocampus length from midline [mm]	2.678±0.802	3.236±0.466	3.347*±0.305	2.364±0.278
Hippocampus length dentate gyrus [mm]	1.184±0.326	1.175±0.084	1.213±0.156	1.021±0.177
Cerebellum height [mm]	4.324±0.253	4.393±0.178	4.383±0.252	4.217±0.387
Cerebellum length [mm]	5.903±0.243	5.815±0.345	5.859±0.129	5.549*±0.392
Cerebellum preculminate fissure thickness of inner granular layer [µm]	128.828±32.451	139.554±27.198	136.777±29.745	144.277±21.285
Cerebellum preculminate fissure thickness of molecular layer [µm]	159.382±17.968	165.465±26.671	165.557±21.801	181.588±28.272
Cerebellum prepyramidal fissure thickness of inner granular layer [µm]	112.689±8.671	122.105±17.676	120.788±21.483	121.926±22.100
Cerebellum prepyramidal fissure thickness of molecular layer [µm]	153.100±13.628	161.226±17.085	157.497±9.761	157.577±23.822

a Data from Table 21 (pages 154-179) of the study report.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study report concluded that the No Observed Effect Level (NOEL) was 20 ppm [1.8 mg/kg/day] in male pups. The Lowest Observed Effect Level (LOEL) in male pups was 75 ppm [6.9 mg/kg/day], based on a

slight reduction in pup body weights, reductions in startle amplitude in the auditory startle test for females on day 23, and treatment-related reductions in some morphometric measurements in areas of the cerebellum of the brain at day 12 only. In female pups at 20 ppm, the only finding was a reduction in startle amplitude in the auditory startle test on day 23, with a lack of an effect on day 63. It was stated that the changes in female pups at 20 ppm and in pups [both sexes] at 75 ppm were completely reversible, indicating that they represent a developmental delay rather than irreversible developmental neurotoxicity. Therefore, the investigator concluded that the 75 ppm dose level administered to dams during gestation and lactation represents a No-Observed-Adverse-Effect-Level [NOAEL].
The investigator concluded that maternal toxicity had been demonstrated at 300 ppm, as evidenced by the reductions in body weight and food consumption. No clinical signs of toxicity or treatment-related macroscopic *post mortem* findings were noted in the dams.

B. REVIEWER'S DISCUSSION

MATERNAL TOXICITY: There was no evidence of a treatment-related effect on maternal survival, and there were no clinical signs of toxicity observed. At 300 ppm [the highest dose tested], there was a slight decrease in maternal body weight from gestation day 10 and throughout lactation compared to the controls. Mean body-weight gain at the 300 ppm dose level was decreased prior to dosing and during gestation days 7-22. During the first 3 days of dosing, dams at 300 ppm displayed a negative body-weight gain. During lactation, the 300 ppm dose group displayed a negative body-weight gain during days 1-7, and the overall body-weight gain for both the 75 ppm and 300 ppm dose groups was decreased [dose-related] compared to the control. At the high-dose level, there was a significant decrease in food consumption throughout gestation and lactation.

Litter size and the number of pups born live/dead were comparable among the groups, and the mean number of total pups born and live birth index were unaffected by treatment. The mid- and high-dose groups displayed the lowest percent of litters with all pups born live compared to the controls. At 300 ppm, there was an increase in the number of litters with small female pups, a slightly higher mortality rate during days 1 to 5 *post partum*, and the number of missing and presumed dead pups [both sexes] was increased compared to the controls. Whole litter losses occurred at the control [2 litters] and high-dose [4 litters] levels only.

There were no treatment-related findings reported in the dams at necropsy [brain weights were not measured].

SELECTED F1 OFFSPRING: For the selected F1 pups, increased preweaning mortality was observed at 300 ppm and a higher number of 300 ppm pups were reported missing and presumed dead compared to the controls. There was a significant decrease in birth weight and an increase in the number of small pups of both sexes at 300 ppm compared to the control group. There was no effect on the sex ratio [percent males].

Decreased body weight was observed at the 300 ppm dose level for both sexes from day 5-29 of lactation, and the decrease continued post weaning [days 29-63], although the magnitude of the decrease in both sexes decreased with time. Decreased body-weight gains were observed mainly during the preweaning period in both sexes at 300 ppm.

There was a delay in both preputial separation and vaginal opening at 300 ppm compared to the control groups. On day 23 *post partum*, there was a significant decrease in the startle amplitude for both sexes at 300 ppm at all 5 intervals, and the females at this time point displayed a dose-related decrease in the startle

amplitude, which was statistically significant at all dose levels in 3 of 5 intervals. Males at all dose levels and females at the low- and mid-dose levels displayed comparable responses to those of the controls on day 61, but the high-dose females continued to display a decrease in startle amplitude on day 61. Time to maximum amplitude was increased on day 23 in the high-dose males only and only during the second interval. On day 61, females at 300 ppm displayed a significant increase in the time to maximum amplitude during 4 of the 5 intervals. Motor activity was comparable among the female groups, but an effect on this parameter cannot be ruled out for males at the 300 ppm dose level because of the initial [day 14] decrease and subsequent, sustained [days 22 and 60 *post partum]*, increase in motor activity observed. Straight-channel swimming time was increased at 300 ppm in both sexes on day 21 *post partum* compared to the controls but comparable among the groups at all other time points. In both the initial learning [day 21] and memory [day 24] phases of the Y-shaped water maze test, both sexes at 300 ppm had a lower percentage of successful trials compared to the controls throughout the test. In the subsequent learning [day 59] and memory [day 62] phases of the Y-shaped water maze test, comparable successes were observed among the groups [both sexes].

There was a treatment-related decrease in absolute brain weight for both sexes at 300 ppm at both the day 12 and day 63 sacrifice times. Brain length was decreased in both sexes at 300 ppm on day 12, and the females of this group also displayed a decrease in brain width. At day 63, slight decreases in both length and width were observed in both sexes at 300 ppm, but statistical significance was not attained.

There were no treatment-related findings at necropsy on either day 12 or day 63, no microscopic abnormalities in the brains of any pups on day 12, and there were no changes in the central or peripheral nervous systems on day 63 that could be attributed to treatment. With respect to morphometric measurements, treatment–related changes in the cortex and/or cerebellum of the brain [decreased structural measurements and decreased thickness of cellular layers] were observed at the mid- and high-dose levels on day 12, and similar treatment-related changes in the cortex, hippocampus, and/or cerebellum were observed at the 300 ppm dose level on day 63.

The NOEL for maternal toxicity is 75 ppm [6.9 mg/kg/day], and the LOEL for maternal toxicity is 300 ppm [26.1 mg/kg/day], based on decreased body weight/gain and food consumption.

The NOEL for developmental neurotoxicity is 20 ppm [1.8 mg/kg/day], and the developmental LOEL is 75 ppm [6.9 mg/kg/day], based on a reduction in startle amplitude in the auditory startle test in females [day 23] and treatment-related reductions in some morphometric measurements in areas of the cerebellum of the brain [day 12] in both sexes. At 300 ppm [26.1 mg/kg/day], (1) increased mortality, (2) decreased body weight, (3) a delay in the appearance of developmental landmarks [preputial separation and vaginal opening], (4) an increase in swimming time in the straight channel test at day 21 and reduced performance in the learning and memory tests on days 21 and 24, respectively, (5) a reduction in startle amplitude, (6) an increase in the time to maximum amplitude [days 23 and/or 61], (7) a possible increase [slight] in mean motor activity level in males, (8) reduced brain weight [both sexes on days 12 and 63], brain length [both sexes on day 12], and brain width [females on day 12], and (9) reductions in several morphometric measurements in areas of the cortex, hippocampus, and cerebellum of the brain were observed.

C. STUDY DEFICIENCIES

The study deviates from the Developmental Neurotoxicity Study Guidelines (OPPTS 870.6300) in the following areas:

(1) Body-weight gains were not provided; (2) Sex ratio was not provided; (3) Two sections of brain from the morphometry analysis were not reported for day 63 [cerebellum-prepyramidal fissure-thickness of outer granular layer and cerebellum-preculminate fissure-thickness of outer granular layer]; (4) The figure on page 198 [Appendix F] is mislabeled; it should be Figure G not Figure E.

None of these adversely affects interpretation of the results.

D. NEUROTOXICITY FINDINGS IN OTHER MOLINATE STUDIES

Neurotoxicity has been a consistent finding in acute, subchronic, and chronic toxicity studies on Molinate in multiple species [Table 20].

[MOLINATE] DEVELOPMENTAL NEUROTOXICITY STUDY §83-6; OPPTS 870.6300

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