WASHINGTON, D.C. 20460

(3)

TXR: **0052378**

MEMORANDUM

DATE: August 31, 2005

SUBJECT: **NALED: Data Evaluation Record of a Developmental Neurotoxicity Study**

PC Code: 034401
DP Barcode: D298945
Reregistration Case #: 0092

FROM: Jessica Kidwell
Science Information Management Branch
Health Effects Division (7509C)

TO: Tom Myers
Risk Manager 52
Reregistration Branch 2
Special Review & Reregistration Division (7508C)

THRU: Brenda May, Branch Senior Scientist
Science Information Management Branch
Health Effects Division (7509C)

I. Action Requested

Review/prepare a Data Evaluation Record for a Developmental Neurotoxicity Study with Naled (MRID Nos. 46153102 and 46153101).

II. Conclusions

Attached is the Data Evaluation Record for a Developmental Neurotoxicity (DNT) Study with Naled (MRID Nos. 46153102 and 46153101). This study is classified Acceptable/Non Guideline and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426 (draft)) due to the need for additional brain morphometric data as well as pending a comprehensive review of all available positive control data. This classification scheme is applicable only to the Developmental Neurotoxicity studies as determined by the DNT Work Group.

SEP 13 2005

DATA EVALUATION RECORD

NALED

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

Work Assignment No. 1-01-25 (MRIDs 46153102 and 46153101)

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

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

Primary Reviewer
David A. McEwen, B.S.

Signature: David A. McEwen
Date: 6/1/04

Secondary Reviewer
Michael E. Viana, Ph.D.

Signature: Michael E Viana
Date: 6/1/04

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/1/04

Quality Assurance
Steve Brecher, Ph.D.

Signature: Steven Brecher
Date: 6/1/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica Kidwell
SIMB, Health Effects Division (7509C)
EPA Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: Jessica Kidwell
Date: 8/31/05
Signature: PV Shah
Date: 8/31/05
Template version 11/01

DATA EVALUATION RECORD

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

PC CODE: 034401
TXR#: 0052378

DP BARCODE: D298945
SUBMISSION NO.: None

TEST MATERIAL (PURITY): Naled (95.4% a.i.; Lot #: 1120040067)

SYNONYMS: 1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate

CITATION: Moxon, M.E. (2003) Naled: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Study Id.: RR0882, October 8, 2003. MRID 46153102. Unpublished

Moxon, M.E. (2003) Naled: Preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Study Id.: RR0881, November 10, 2003. MRID 46153101. Unpublished

SPONSOR: AMVAC Chemical Corporation, 4100 East Washington Blvd., Los Angeles, CA

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRID 46153102), Naled (95.4% a.i.; Lot #: 1120040067) in dried corn oil was administered to pregnant Alpk:AP$_f$SD Wistar-derived rats (30/dose) daily via gavage (10 mL/kg) from gestation day (GD) 7 to lactation day (LD) 7 at doses of 0, 0.4, 2, or 10 mg/kg/day. Additionally, the F_1 pups were dosed orally by gavage with 0, 0.4, 2 or 10 mg/kg/day on postnatal days (PNDs) 8-22. Dams were allowed to deliver naturally and were killed on LD 29. The dams were subjected to a functional observational battery (FOB) on GDs 10 and 17 and LDs 2 and 9. On PND 5, litters were standardized to 8 pups/litter; the remaining offspring and dams were sacrificed and discarded without further examination. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) were allocated to subsets for the following investigations: FOB (PNDs 5, 12, 22, 36, 46, and 61), motor activity (PNDs 14, 18, 22, and 60), acoustic startle response (PNDs 23 and 61), learning and memory evaluation (PNDs 24 and 27 and PNDs 59 and 62), and postmortem observations including brain weight, neuropathology and morphometry (PND 12 and 63). Dosing was based on a preliminary developmental neurotoxicity study in rats (MRID 46153101).

In dams, no treatment-related effects on mortality, clinical signs, FOB, body weight, reproductive performance, or gross pathology parameters were observed at any dose.

The maternal NOAEL is 10 mg/kg/day (highest dose tested). The maternal LOAEL is not established.

Treatment had no adverse effects on offspring survival, clinical signs, FOB, body weight, body weight gain, developmental landmarks, learning and memory, or neuropathology.

A treatment-related decrease in total motor activity (not statistically significant) was seen in F1 males at 2 and 10 mg/kg/day on PND 14 (↓46%, ↓49%, respectively) and PND 18 (↓36%, ↓50%, respectively). [It is noted that the data are highly variable (large standard deviations) for the control and treated groups such that statistical significance was not attained.] The total mean number of movements were increased between PND 14 and 22 in all groups. In the male sub-session data, there was a dose-related decrease in motor activity ($p \leq 0.05$) at 2 mg/kg (↓78%) and 10 mg/kg/day (↓90%) on PND 18 during the 41-45 minute sub-session. These findings are consistent with the overall decrease in motor activity seen on PND 14 and 18 in males at ≥2 mg/kg/day. In addition, the repeated dose cholinesterase studies showed biologically significant decreases in brain cholinesterase activity (↓14-42%) for PND 18 males at the same doses where effects in motor activity were seen in the DNT study.

In males, a consistent decrease (not statistically significant, high variability) in mean auditory startle reflex peak amplitude was noted at the high dose (10 mg/kg/day) at PND 23 (↓13-26%) and PND 61 (↓10-31%) that may be attributable to treatment. No treatment-related differences were observed in latency of auditory response in males. No treatment-related differences were observed in peak amplitude or latency of auditory startle response in females.

Absolute brain weights were statistically significantly decreased (↓6%, $p < 0.05$) in females only at the high dose (10 mg/kg/day). Also at the high dose, several brain morphometric measurements were significantly different ($p \leq 0.05$) from the concurrent controls: (i) increased thickness of the dorsal cortex in the males on PND 12 (↑5%); (ii) increased width of the dentate gyrus and overall width in the hippocampus in the males on PND 12 (↑6-9%); (iii) decreased length of the dentate gyrus in the hippocampus in the females on PND 12 (↓15%); (iv) increased length from the midline and length of the dentate gyrus in the hippocampus in the males on PND 63 (↑9-11%); and (v) increased width of the dentate gyrus in the hippocampus in the females on PND 63 (↑8%).

Due to a variety of statistically significant effects seen in the brain morphometric data both in both males and females, examination of the following brain regions from the mid and low dose groups are requested: dorsal cortex, hippocampus, and prepyramidal fissure of the inner granule layer of cerebellum.

3

The offspring LOAEL is 2 mg/kg/day, based on decreased total motor activity in males at PND 14 and 18 and decreased subsession motor activity in males in the 41-45 minute interval on PND 18. The offspring NOAEL is 0.4 mg/kg/day.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426 (draft)) due to the need for additional brain morphometric data as well as pending a comprehensive review of all available positive control data.

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

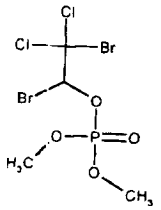
4

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Naled

Description:	Off-white to straw yellow clear viscous liquid
Lot #:	1120040067
Purity:	95.4% a.i.
Compound Stability:	The test substance was stable in the vehicle for up to 15 days at room temperature.
CAS # of TGAI:	300-76-5
Structure:	

2. Vehicle and/or positive control - Dried corn oil

3. Test animals (P)

Species:	Rat	
Strain:	Alpk:AP$_f$SD (Wistar-derived)	
Age at GD 1:	10-12 weeks	
Wt. at GD 1:	214-291 g (females)	
Source:	Rodent Breeding Unit (RBU), Alderly Park, Macclesfield, Cheshire, UK	
Housing:	P dams were housed individually until parturition and then with their litters until PND 29 in solid plastic cages with wood shaving bedding. F_1 animals were housed 4/cage (separated by sex) in wire mesh cages.	
Diet:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum*, except during behavioral testing	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70%
	Air changes:	≥15/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. In-life dates - Start: August 20, 2002 End: August 1, 2003

2. Study schedule - The test substance was administered to the maternal animals from gestation day (GD) 7 through lactation day (LD) 7 and to the F_1 pups from post-natal day (PND) 8 through 22. F_1 pups were selected on PND 5, separated from dams on PND 29, and assigned to subgroups in order to evaluate behavioral abnormalities, motor activity, auditory startle response, learning and memory, and neuropathology, including morphometry and brain weights.

3. **Mating procedure** - The maternal animals were mated by the supplier and examined for the presence of spermatozoa in a vaginal smear to verify positive mating. Dams were shipped to the performing laboratory the day on which positive mating was found, designated GD 1. Twenty time-mated females were supplied on each of 6 days.

4. **Animal assignment** - Mated females were randomly assigned, blocked by arrival day, to dose groups as indicated in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 5.

Table 1. Study design [a]

Parameter	Dose (mg/kg)			
	0	0.4	2	10
Maternal Animals				
No. of maternal animals	30	30	30	30
FOB (GD 10 & 17; LD 2 & 9)	30	30	30	30
Offspring				
FOB [b] (PND 5, 12, 22, 36, 46, & 61)	11-13/sex/litter	10-12/sex/litter	11-12/sex/litter	10-13/sex/litter
Motor activity [b] (PND 14, 18, 22, & 60)	10-13/sex/litter	10-13/sex/litter	10-13/sex/litter	10-13/sex/litter
Auditory startle test (PND 23 & 61)	10-12/sex/litter	10-12/sex/litter	10-12/sex/litter	10-12/sex/litter
Learning and memory (water maze) (PND 24 & 27) (PND 59 & 62)	19-24/sex/litter 22-24/sex/litter	19-24/sex/litter 22-24/sex/litter	19-24/sex/litter 22-24/sex/litter	19-24/sex/litter 22-24/sex/litter
Brain and cerebellum weight only (PND 12 & 63)	1pup/sex/litter (10 pups/sex)	1pup/sex/litter (10 pups/sex)	1pup/sex/litter (10 pups/sex)	1pup/sex/litter (10 pups/sex)
Brain & cerebellum weight and neuropathology	10 pups/sex	10 pups/sex	10 pups/sex	10 pups/sex
Brain cholinesterase determination	NA	NA	NA	NA

a Data obtained from pages 19-24 of MRID 46153102.
b The same animals were used for both FOB and motor activity testing.
NA Not applicable

5. **Dose selection rationale** - Dose levels were chosen based on the results of a preliminary developmental neurotoxicity study in the rat (MRID 46153101), submitted concurrently, and a repeated-dose cholinesterase inhibition study in pre-weaning and young adult rats (MRID 46153104). A summary of the range-finding study is included as Appendix I in this DER. In the range-finding study, naled was administered by gavage to pregnant Wistar rats from GD7 to LD 22 at doses of 0, 3, 10, or 30 mg/kg/day. Dose levels of 10 and 30 mg/kg/day were associated with maternal toxicity, including transient decreases in body weight and inhibition of brain and erythrocyte cholinesterase activities. The dose level of 3 mg/kg/day was associated with a

reduction in erythrocyte cholinesterase activity in maternal animals. The dose level of 30 mg/kg/day was associated with transient decreases in F1 body weight and reduced brain and erythrocyte cholinesterase activities in fetuses and pups. No effects were seen at 3 or 10 mg/kg/day in the offspring. In repeated-dose cholinesterase inhibition study, naled was administered daily via gavage to groups of 5 rats/sex from PND 12-18 or 42-48 at doses of 0, 0.4, 2, or 10 mg/kg and 0 and 30 mg/kg/day. At 10 mg/kg, both erythrocyte and brain cholinesterase activities were decreased in the pre-weaning rats, while only erythrocyte cholinesterase activity was decreased in the young adult rats. Brain cholinesterase activities were decreased in young adult rats at 30 mg/kg/day. The NOAELs/LOAELs for RBC and brain cholinesterase activities are as follows:

Adult RBC ChE inhibition NOAEL =2 mg/kg/day; LOAEL = 10 mg/kg/day based on 31-40% decrease in RBC ChE in males and females

Adult Brain ChE inhibition NOAEL = 10 mg/kg/day; LOAEL = 30 mg/kg/day based on 57-66% decrease in Brain ChE in males and females

Pre-weaning RBC ChE inhibition NOAEL = not identified; LOAEL = 0.4 mg/kg/day based on 22% decrease in RBC ChE in PND 18 females

Pre-weaning Brain ChE inhibition NOAEL = 0.4 mg/kg/day; LOAEL = 2 mg/kg/day based on 14% decrease in Brain ChE in PND 18 males

Therefore, the doses selected for the main DNT study (0, 0.4, 2 and 10 mg/kg/day) were adequate.

6. **Dosage administration** - All doses were administered daily via gavage (10 mL/kg) to the maternal animals from GD 7 through LD 7 and to the F_1 pups from PND 8 through 22.

7. **Dosage preparation and analysis** - Dosing formulations were prepared weekly by adding the appropriate amount of dried corn oil to a weighed amount of the test material to make the highest treatment dose. The lower doses were prepared by serial dilution of the highest concentration. Each batch was divided into aliquots for daily use and stored refrigerated, under nitrogen. Concentration analyses were performed on formulations from all dose groups in three batches prepared at the beginning, middle, and end of the dosing period. Additionally, stability after 15 days at room temperature was verified for the 0.04 and 10 mg/mL formulations. It was stated that because the dose preparations were solutions, homogeneity was not determined.

Results - **Stability:** 100.9-106.8% initial concentration after 15 days at room temperature.

Concentration: 100-110% nominal

The analytical data indicated that the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals - Cage-side observations were conducted at least once daily. Detailed clinical examinations were performed at the same time that body weights were measured. On dosing days, observations were conducted prior to dosing.

All rats were examined outside the home cage on GD 10 and 17 and LD 2 and 9 using a functional observation battery which included, but was not limited to, the following:

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection 3) Urination and defecation 4) Ptosis 5) Exophthalmos 6) Pupillary function
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, and general signs of toxicity (thin, altered muscle tone, dehydrated, or altered fur appearance).

Body weights were recorded on: GD 1; immediately prior to dosing on GD 7 through LD 7; LD 15, 22, and 29; and at termination. Food consumption was not reported.

b. Offspring

1) Litter observations - Each litter was examined as soon as possible (always within 24 hours) after completion of parturition (PND 1). The sex of each pup was recorded on PND 1 and 5. The body weight and clinical condition of each pup was recorded on PND 1 and 5 (post-cull), and immediately prior to dosing on PND 8 through 22. In addition, daily checks for dead or abnormal pups were conducted.

On PND 5, litters were standardized to 8 pups (4/sex/litter, as near as possible), and selection of the F_1 generation was carried out using litters comprised of 7 or 8 pups with at least 3 males and 3 females. Pups not selected for further evaluations on PND 5 were killed and discarded.

2) Developmental landmarks - Beginning on PND 29, the selected F_1 females were examined daily to determine the age at which vaginal opening occurred. Beginning on PND 36, the selected F_1 males were examined daily to determine the age at which balanopreputial separation occurred.

3) **Postweaning observations** - Cage-side observations for mortality and clinical signs of toxicity were performed daily. Body weight measurements and detailed physical examination of each rat was conducted on PND 29, 36, 43, 50, 57, and prior to termination on PND 63. Body weights on the day of balanopreputial separation/vaginal patency were recorded.

4) **Neurobehavioral evaluations**

i) **Functional observational battery (FOB)** - At least 10 rats/sex/dose (one male or one female per litter) were examined on PND 5, 12, 22, 36, 46, and 61 using a functional observational battery comprised of the same parameters examined in the maternal rats. It was not stated if the FOB was conducted by a technician who was "blind" to the treatment groups. Detailed FOB procedures were not provided in the study report.

ii) **Motor activity testing** - Motor activity was evaluated in at least 10 rats/sex/dose (one male or one female per litter) on PND 14, 18, 22, and 60, using an automated recording apparatus (details not provided) which recorded small and large movements as an activity count. Each 50 minute session was divided into 10 subsessions of five-minute duration. The same offspring were evaluated at each time point, and each animal was returned to the same activity monitor when trials were repeated. Motor activity testing was performed in a separate room to minimize disturbance. The treatment groups were counter-balanced across cage/device numbers.

iii) **Auditory startle reflex habituation** - An auditory startle habituation test was conducted on at least 10 rats/sex/dose (one male or one female per litter) on PND 23 and 61, using an automated recording apparatus (details not provided). Fifty trials (repetitions) were performed on each animal per each day of testing. The mean response amplitude and time to maximum amplitude were calculated on each block of 10 trials (5 blocks of 10 trials per day of testing).

iv) **Learning and memory testing** - Associative learning and memory were tested in one rat/sex/litter/dose on PND 24 and 27 and another rat/sex/litter/dose on PND 59 and 62. The test used a Y-shaped water maze with one escape ladder. The time taken to find the escape ladder was recorded for each trial. Animals were given 6 trials on either PND 24 or 59 (learning phase) and were retested three days later (PND 27 or 62) using the same procedures (memory phase). In order to assess swimming speed, each animal completed one trial in a straight channel immediately following the six trials during the learning phase and the memory phase. Swimming times for the Y-maze and straight channel were reported. The percentage of successful trials in the Y-maze was calculated for each animal. The criterion for a successful trial was a time less than a given cut-off value. Cut-off values of 3, 4, 5, 6, 7, 8, 9, and 10 seconds and 1x, 1.5x, and 2x the straight channel time were used.

5) **Cholinesterase determination** - Cholinesterase activity was not determined.

6) **Pharmacokinetic data** - Pharmacokinetics were not evaluated in this study.

2. Postmortem observations

a. Maternal animals - Any females that did not litter or showed signs of difficult parturition were killed by over exposure to halothane Ph. Eur. vapor followed by exsanguination and were subjected to a macroscopic examination (including an examination of the uterus to confirm pregnancy status). Females with total litter loss or with litters not required for selection were sacrificed and discarded. On PND 29, all surviving maternal animals were sacrificed and discarded without examination.

b. Offspring - Prior to selection on PND 5, pups found dead or killed *in extremis* were discarded without examination. On PND 12, at least 10 pups/sex/group (one male or one female per litter) were killed by a rising concentration of carbon dioxide. The brain and cerebellum were immediately fixed in formol saline and weighed after at least 24 hours fixation. The brains from the control and 10 mg/kg groups were embedded in paraffin, sectioned into 7 levels, and stained with hematoxylin and eosin.

On PND 63, at least 10 pups/sex/group (one male or one female per litter) were killed as described for PND 12, except that after weighing, the brain and cerebellum were fixed and stored. A further 10 rats/sex/group were deeply anesthetized by intraperitoneal injection of sodium pentobarbitone and killed by perfusion fixation with formol saline. The brain and cerebellum were removed and weighed, and the following CHECKED (X) tissues were collected and preserved in an appropriate fixative.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Frontal cortex	X	Sciatic nerve (proximal)
X	Dorsal cortex		
X	Piriform cortex		**OTHER**
X	Hippocampus		Sural nerve
X	Corpus callosum	X	Tibial nerve (proximal and distal)
X	Thalamus		Peroneal nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
	SPINAL CORD	X	Lumbar dorsal root fibers
X	Cervical swelling	X	Lumbar ventral root fibers
X	Lumbar swelling	X	Cervical dorsal root ganglion
	OTHER	X	Cervical dorsal root fibers
	Gasserian ganglia with nerve	X	Cervical ventral root fibers
	Pituitary gland		
X	Eyes (with retina and optic nerve)		
X	Skeletal muscle (gastrocnemius)		

The tibial and sciatic nerves were embedded in resin, sectioned, and stained with toluidine blue. The remaining tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues from all animals were processed, and the tissues from the control and 10 mg/kg groups were subjected to neuropathological examination, including morphometric analysis, using light microscopy. Additionally, the number of Purkinje cells in lobe 8 adjacent to the prepyramidal fissure were counted and expressed as a function of the length of lobe 8 from lobes 1 and 4. The number of Purkinje cells/mm was calculated for each animal.

D. DATA ANALYSIS

1. Statistical analyses - Data were analyzed using the following statistical procedures:

Parameter	Statistical test
Maternal body weights	Body weights on LD 1 were analyzed by analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used for body weights during gestation using GD 7 body weight as the covariate and for body weights during lactation (other than LD1) using LD 1 as the covariate. ANOVA (or ANCOVA) were followed by Student's t-test, if necessary, for pair-wise comparison of treated groups with controls.
Pup body weight	Body weights on PND 1 and those selected on PND 5 were analyzed by ANOVA. Body weights on subsequent pre-cull days and post-cull days were adjusted for body weights on PND 1 or PND 5, respectively, and analyzed using ANCOVA. ANOVA (or ANCOVA) were followed by Student's t-test, if necessary, for pair-wise comparison of treated groups with controls.
Litter size, motor activity, startle response, gestation length, litter weight, time to preputial separation, time to vaginal opening, swimming times	ANOVA followed by Student's t-test, if necessary.
Proportions of whole litter loss, pups born live, pups surviving, litters with all pups born live, litters with all pups surviving, male pups, males with preputial separation, females with vaginal opening, litters with gestation length ≥ 22 days	Fisher's Exact Test
Percentages of live born pups, pre-cull pup survival, sex ratio, and successful swimming trials	ANOVA following the double arcsine transformation of Freeman and Tukey, followed by Student's t-test, if necessary.
Brain and cerebellum weights and brain morphology	ANOVA and ANCOVA using the terminal body weight as the covariate followed by Student's t-test, if necessary.
Number of Purkinje cells	ANOVA, separately by sex

Analyses of lactation (post-natal) body weights, litter size, and pup survival are presented excluding whole litter losses. Analyses of live born index are presented including and excluding whole litter losses. All statistical tests were two-sided.

11

2. Indices - Although the formula was not provided, live birth index was reported as the percent of live born pups including whole litter losses. No other indices were reported. The reviewers calculated the following indices and included the data in the summary tables.

Gestation index (%) = # dams with live pups on the day of birth/# pregnant x 100

Viability index (%) = # of pups surviving to PND 5 (pre-cull)/# of pups born live x 100

3. Positive control data - Positive control data (2003-2004) from Central Toxicology Laboratory (CTL) on brain morphometry in pups (MRID 46336204) and on FOB, motor activity and morphometry in adult rats, both studies using trimethyltin chloride, (MRID 46336203) were submitted and are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations** - No treatment-related deaths occurred. The following dams (1 rat/dose) were found dead or sacrificed prior to scheduled termination: (i) 0.4 mg/kg on LD 6, clinical signs indicative of dosing error; (ii) 2 mg/kg on LD 1, clinical signs associated with parturition; and (iii) 10 mg/kg on LD 1, found dead with signs of difficult parturition (one dead fetus protruding from the vagina causing vaginal intussusception). Several other dams (control and treated) were sacrificed because they did not litter, suffered complete litter loss, or because there were insufficient numbers of pups (required at least 7 pups with at least 3 pups/sex/litter). However, these findings were not dose-related.

There were no treatment-related clinical or functional observations.

2. **Body weight and food consumption** - There were no treatment-related effects on body weights or body weight gains during gestation (Table 2). Only minor and transient increases (↑1%; $p \leq 0.05$) in body weights were observed in the 0.4 and 10 mg/kg dams on GD 8, 10, and 17. During lactation, minor decreases ($p \leq 0.05$) in body weights (↓2% each) were observed in the 2 mg/kg dams on LD 3, and in the 10 mg/kg dams on LD 3-5. Body weight gain for the overall lactation period (LD 1-29) was similar to controls. Food consumption was not reported.

Table 2. Mean (± SD) maternal body weights (g) and body weight gains (g) in dams treated with Naled via gavage on GD 7 to LD 7. [a]

Interval (Days)	Dose (mg/kg)			
	0	0.4	2	10
Gestation (n=28-30)				
1	255.3±21.7	255.8±17.3	255.6±17.7	254.9±15.9
8[b]	292.2	294.0* (↑1)	292.7	293.6
10[b]	300.4	301.5	301.3	303.2* (↑1)
17[b]	354.8	356.8	358.7	359.8* (↑1)
22[b]	423.4	422.7	427.9	431.4
Overall body weight gain (GD 1-22)[c]	172.0	165.6	171.4	175.4
Lactation (n=22-30)				
1	332.2±25.7	332.2±24.0	335.5±26.3	338.7±28.2
3[b]	338.9	338.3	333.6* (↓2)	333.4* (↓2)
5[b]	342.6	342.7	339.1	335.9* (↓2)
22[b]	354.7	355.7	357.3	357.6
29[bd]	340.0	342.2	341.2	342.6
Overall body weight gain (LD 1-29)[c]	4.1	9.3	4.3	5.0

a Data obtained from Tables 5 & 6 on pages 71-75 in MRID 46153102. Percent difference from controls, calculated by the reviewers, is included in parentheses.

b Adjusted means

c Calculated by the reviewers from the differences in unadjusted group mean body weights.

d Post-weaning

* Statistically different from the controls at $p \leq 0.05$.

13

3. **Reproductive performance** - Reproductive performance was unaffected by the test substance (Table 3).

Table 3. Reproductive performance [a]

Observation	Dose (mg/kg)			
	0	0.4	2	10
Number mated	30	30	30	30
Number pregnant	29	30	30	30
Number not pregnant	1	0	0	0
Number of litters (# with live born)	28	30	30	29
Gestation index (%) [b]	96.6	100	100	96.7
Number with complete litter loss	1	0	1	1
Insufficient number or sex ratio of pups [c]	3	7	4	3
Mean (±SD) gestation duration (days)	22.0±0.0	22.0±0.0	22.0±0.2	22.0±0.0
Incidence of dystocia	0	0	0	1

a Data obtained from pages 27 and 76 in MRID 46153102.

b Gestation index was calculated by the reviewers from data presented in this table as: # with live born/ # pregnant x 100.

c Sufficient number of pups was defined as at least 3 males and 3 females in a litter of at least 7 pups. Dams and litters not meeting this criteria were sacrificed.

4. **Maternal postmortem results** - No treatment-related findings were noted at necropsy.

B. OFFSPRING

1. **Viability and clinical signs** - Litter size and viability data for pups prior to selection on PND 5 are summarized in Table 4. Litter size and survival data were not presented after culling on PND 5. However, it was stated that a small number of F_1 pups failed to survive to scheduled termination. No treatment-related findings were noted.

There were no treatment-related clinical signs.

Table 4. Litter size and viability [a]

Observation		Dose (mg/kg)			
		0	0.4	2	10
Total number born		346	348	381	361
Number born live		345	346	376	358
Number born dead [b]		1	2	5	3
Live birth index (%, including whole litter loss)		99.8	99.0	98.6	98.9
Sex Ratio Day 1 (% ♂)		46.9	51.1	49.4	50.9
Mean litter size:	Day 1	12.3±3.1	11.5±3.4	12.4±3.0	12.7±3.1
	Day 5 [c]	11.9±2.6	11.3±3.2	11.9±2.9	12.3±3.1
	Day 5 [d]	NR	NR	NR	NR
	Day 21	NR	NR	NR	NR
Viability index (%) [e]		92.8	97.7	88.8	96.4
Lactation index (%)		NR	NR	NR	NR

a Data obtained from Tables 8 through 12 on pages 77-81 in MRID 46153102.
b Calculated by the reviewers from data presented in this table.
c Before standardization (culling).
d After standardization (culling).
e Calculated by the reviewers as # surviving PND 1-5 (found in Table 11 on page 80 of MRID 46153102) divided by # born live (found in this table) x 100.
NR Not reported

2. **Body weight** - No treatment-related effects on pre-weaning (Table 5a) or post-weaning (Table 5b) pup body weights were observed in either sex. The minor increases (↑2-3%; $p \leq 0.05$) noted in the 10 mg/kg males on PND 9-11 and females on PND 19 were not adverse.

Table 5a. Mean (±SD) pre-weaning pup body weights (g) [a]

Post-natal Day	Dose (mg/kg)							
	0	0.4	2	10	0	0.4	2	10
	Males				Females			
1	6.1±0.5	6.2±0.6	6.0±0.5	6.3±0.7	5.7±0.4	5.8±0.6	5.7±0.4	5.9±0.6
5 [b,d]	9.9	9.7	9.5	9.7	9.4	9.3	9.1	9.2
5 [c]	9.8±0.9	9.6±1.3	9.4±0.8	9.6±1.1	9.4±0.9	9.1±1.1	9.1±0.9	9.2±1.0
9 [e]	17.4	17.6	17.7	17.8* (↑2)	16.8	16.8	16.9	17.1
11 [e]	21.9	22.3	22.3	22.5* (↑3)	21.2	21.5	21.6	21.7
19 [e]	43.1	44.3	43.3	44.3	41.6	42.3	42.1	42.9* (↑3)
22 [e]	53.7	55.3	53.9	55.4	52.1	53.1	52.6	53.5

a Data were obtained from Tables 13 and 17 on pages 82 and 118-123 in MRID 46153102; n=22-30. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Before standardization (culling)
c After standardization (culling)
d Means are adjusted based on the body weight on PND 1.
e Means are adjusted based on the body weight on PND 5 (post-cull).
* Statistically different from controls at $p \leq 0.05$

Table 5b. Adjusted mean post-weaning pup body weights (g) [a]

Post-natal Day	Dose (mg/kg)							
	0	0.4	2	10	0	0.4	2	10
	Males				Females			
29	91.6	93.2	91.3	92.0	86.4	86.8	86.3	87.0
50	253.5	258.6	255.0	252.9	189.4	189.8	189.3	189.3
63	344.4	347.6	342.7	341.9	220.5	222.9	221.3	219.1

a Data were obtained from Table 17 on pages 120 and 123 in MRID 46153102. Means are adjusted based on the body weight on PND 5 (post-cull). Percent difference from controls, calculated by the reviewers, is included in parentheses.

3. Developmental landmarks

a) Sexual maturation - Sexual maturation data are presented in Table 6. Increased ($p \leq 0.05$) mean time to vaginal opening was observed at 10 mg/kg (35.1 days treated vs 34.1 days controls); however, there were no statistically significant differences in the proportion of females with vaginal opening occurring on any particular day, and the range of days which vaginal opening occurred was similar between treated and control animals. Therefore, this finding was not considered to be treatment related. The test material had no effect on time to preputial separation. Body weight at preputial separation was similar between treated and control males. Body weight at vaginal opening was slightly increased (↑4-5%; not statistically significant) in the treated females compared to controls.

Table 6. Mean (±SD) age of sexual maturation (days) [a]

Parameter	Dose (mg/kg)			
	0	0.4	2	10
n (M/F)	24/24	22/22	24/24	25/25
Preputial separation (males)	44.3±1.1	44.1±1.3	44.4±1.5	44.0±1.5
Body weight at landmark (g)	208.8±12.2	210.4±14.3	208.4±16.3	206.0±16.0
Vaginal opening (females)	34.1±1.1	34.8±1.4	35.0±1.8	35.1±1.9* (13%)
Body weight at landmark (g)	115.6±9.7	120.0±9.8	120.4±12.2	121.5±10.7

a Data were obtained from Table 18 on pages 124-125 in MRID 46153102.
* Statistically different from controls at $p \leq 0.05$

b) **Physical landmarks** - Physical landmarks were not evaluated.

4. **Behavioral assessments**

a) **Functional observational battery** - No abnormalities were detected in any of the parameters examined in the functional observational battery at any time point in either sex.

b) **Motor activity** - A treatment-related decrease in total motor activity (not statistically significant) was seen in males at 2 and 10 mg/kg/day on PND 14 (↓46%, ↓49%, respectively) and PND 18 (↓36%, ↓50%, respectively) (Table 7a). The total mean number of movements were increased between PND 14 and 22 in all groups. In the male sub-session data, there was a dose-related decrease in motor activity ($p \leq 0.05$) at 2 mg/kg (↓78%) and 10 mg/kg/day (↓90%) on PND 18 during the 41-45 minute sub-session (Table 7b). These findings are consistent with the overall decrease in motor activity seen on PND 14 and 18 in males at ≥2 mg/kg/day and are considered to be treatment-related. Motor activity was also decreased ($p<0.05$) compared to the control at ≥0.4 mg/kg/day (↓49%, ↓56%, ↓36%, for 0.4, 2, and 10 mg/kg/day, respectively), however, this finding was not considered to be treatment-related as there was no dose-response. There were no significant differences in the sub-session motor activity data in the females (Table 7c). Although an apparent typical pattern of habituation is observed in treated animals on PND 14, 18, and 60, the lack of habituation in control animals (males on PND 18, 22,and 60 and females on PND18 and 22) confounds this observation. Thus, it is difficult to ascertain the effect of compound exposure on habituation, though it is clear that motor activity is decreased.

17

Table 7a. Mean (±SD) motor activity data (total number of movements/50 minutes) [a]

Test Day	Dose (mg/kg)			
	0	0.4	2	10
Males				
PND 14	179.2±300.6 [168%]	192.9±218.4 (↑8%)	96.8±81.5 (↓46%)	91.2±129.4 (↓49%)
PND 18	251.6±263.2 [105%]	198.3±240.5 (↓21%)	161.1±104.7 (↓36%)	126.8±176.2 (↓50%)
PND 22	358.3±282.8 [79%]	264.5±229.5 (↓26%)	355.0±161.2 (↓<1%)	268.9±194.6 (↓25%)
PND 60	621.0±148.8 [24%]	517.5±190.2 (↓17%)	539.9±140.2 (↓13%)	603.3±163.5 (↓3%)
Females				
PND 14	196.2±178.2 [91%]	139.9±120.0 (↓29%)	172.7±234.4 (↓12%)	198.2±237.9 (↑1%)
PND 18	139.8±169.3 [121%]	152.1±90.2 (↑9%)	210.5±253.1 (↑51%)	193.8±262.5 (↑39%)
PND 22	265.3±214.0 [81%]	240.5±135.5 (↓9%)	326.5±237.2 (↑23%)	349.3±228.5 (↑32%)
PND 60	587.8±119.8 [20%]	600.3±86.5 (↑2%)	620.9±116.8 (↑6%)	597.5±89.8 (↑2%)

a Data were obtained from Table 19 on pages 126-133 in MRID 46153102; n=10-13.
Coefficient of Variation, calculated by reviewers. is included in brackets
Percent difference from controls, calculated by reviewers, is included in parentheses.

Table 7b. Mean (± SD) sub-session motor activity in males (# movements/5 minute sub-session)[a]

Interval (min)	Dose (mg/kg)			
	0	0.4	2	10
PND 14				
1-5	31.7±23.7 [75%]	24.4±23.7 (↓23%)	17.1±14.1 (↓46%)	15.4±21.7 (↓51%)
6-10	19.2±33.2	27.7±32.9 (↑44%)	10.7±11.7 (↓44%)	11.3±16.9 (↓41%)
11-15	18.6±31.0	28.8±22.7 (↑55%)	10.6±10.9 (↓43%)	11.5±21.0 (↓38%)
16-20	17.7±35.1	21.1±31.6 (↑19%)	7.2±9.5 (↓59%)	15.9±24.1 (↓10%)
21-25	16.9±38.0 [225%]	12.4±22.1 (↓27%)	7.7±12.2 (↓54%)	6.9±14.7 (↓59%)
26-30	19.2±26.1	19.9±28.1 (↑4%)	12.6±15.5 (↓34%)	4.8±8.2 (↓75%)
31-35	11.7±28.8	14.5±20.7 (↑24%)	13.9±18.8 (↑19%)	7.7±15.6 (↓34%)
36-40	16.2±35.6	15.4±22.9 (↓5%)	9.6±15.2 (↓41%)	6.8±13.5 (↓58%)
41-45	14.1±29.3	17.6±23.1 (↑25%)	4.5±9.9 (↓68%)	6.8±14.0 (↓52%)
46-50	13.9±33.0	11.1±19.6 (↓20%)	2.7±6.8 (↓81%)	4.1±10.7 (↓71%)
PND 18				
1-5	24.0±28.4	22.2±28.1	31.1±30.3	17.3±25.0
6-10	25.1±25.4	19.8±30.1	22.3±23.4	15.7±20.0
11-15	33.4±33.5	21.1±28.5	19.2±16.0	13.5±21.6
16-20	26.3±30.3	16.5±29.1	16.1±16.9	11.0±22.5
21-25	23.0±27.8	20.4±31.3	15.0±15.2	16.8±30.2
26-30	20.4±28.2	20.6±27.7	13.9±21.9	21.6±29.4
31-35	17.2±27.1	21.8±32.6	14.8±17.3	12.8±20.0
36-40	21.9±30.5	18.4±25.5 (↓16%)	10.2±17.2 (↓53%)	7.3±13.9 (↓67%)
41-45	32.8±37.4	19.6±27.7 (↓40%)	7.2±12.2* (↓78)	3.3±6.9* (↓90)
46-50	27.5±35.0	17.9±28.9 (↓36%)	11.4±19.6 (↓41%)	7.4±17.1 (↓73%)
PND 22				
1-5	34.0±28.4	35.4±27.6	36.5±22.0	28.2±21.3
6-10	36.4±29.9	33.1±28.1	36.9±29.6	30.4±23.4
11-15	29.5±37.4	19.3±25.8	37.5±28.5	18.3±23.4
16-20	29.6±35.0	24.2±24.6	20.4±25.2	29.7±30.9
21-25	45.9±31.4	24.1±25.6	30.4±22.7	32.5±27.7
26-30	40.7±23.9	21.0±27.5	27.5±25.9	23.1±27.9
31-35	34.7±33.9	27.4±32.2	42.6±21.0	17.8±26.6
36-40	40.7±31.9	24.6±30.4	47.9±25.6	24.8±29.3
41-45	34.8±37.5	23.2±26.9	40.9±22.9	31.0±24.7
46-50	32.0±33.1	32.2±28.6	34.4±32.5	33.1±27.0

(table continues next page)

Interval (min)	Dose (mg/kg)			
	0	0.4	2	10
	PND 60			
1-5	76.4±14.5	74.7±10.3	77.5±7.8	76.5±7.7
6-10	69.2±11.4	72.8±14.8	70.7±16.1	71.2±14.6
11-15	64.6±22.7	56.9±16.9	66.9±21.8	71.7±19.7
16-20	52.1±29.4	51.4±26.6	53.8±23.9	62.4±30.7
21-25	50.3±26.6	36.1±32.4	48.9±23.6	55.8±26.8
26-30	57.2±21.4	47.4±30.9	48.6±22.2	60.8±17.2
31-35	57.1±22.4	46.2±27.8	52.9±22.1	57.8±15.0
36-40	58.8±26.8	47.2±28.1	46.4±25.8	48.0±25.6
41-45	62.1±25.4	47.8±36.6	41.9±26.5	52.3±25.3
46-50	73.2±17.9	37.0±29.1** (↓49)	32.2±31.5** (↓56)	46.9±34.1* (↓36)

a Data were obtained from Table 19 on pages 126-133 in MRID 46153102; n = 10-13. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

** Statistically different from controls at $p \leq 0.01$

20

Table 7c. Mean (± SD) sub-session motor activity in females (# movements/5 minute sub-session) [a]

Interval (min)	Dose (mg/kg)			
	0	0.4	2	10
PND 14				
1-5	25.2±21.8	25.1±32.5	26.6±29.6	28.1±32.2
6-10	30.6±30.6	13.0±14.2	18.3±28.8	22.9±32.1
11-15	27.4±26.6	17.6±22.2	14.6±26.6	16.6±26.2
16-20	25.8±27.4	15.7±20.1	21.7±35.7	23.5±26.7
21-25	21.3±22.7	10.4±12.8	22.3±25.4	25.3±30.5
26-30	15.1±23.5	17.7±21.2	18.3±25.9	18.9±26.7
31-35	16.9±21.4	10.8±15.4	18.3±28.7	16.1±31.4
36-40	16.1±27.0	15.5±19.5	11.3±22.3	15.8±28.9
41-45	8.3±20.6	9.6±24.9	7.5±22.0	13.2±21.6
46-50	9.5±19.2	4.5±8.4	13.9±27.4	17.8±27.4
PND 18				
1-5	17.1±21.5	15.5±10.2	23.3±36.1	24.4±32.3
6-10	18.2±23.5	20.8±19.3	18.4±27.7	22.6±33.9
11-15	17.2±19.2	21.5±24.9	20.1±28.1	19.8±34.3
16-20	16.8±19.6	16.7±20.8	25.8±32.6	13.1±24.3
21-25	12.2±17.4	14.5±18.4	16.9±26.3	21.3±34.8
26-30	14.0±20.9	8.5±11.9	22.8±28.5	14.9±33.4
31-35	10.2±19.1	13.7±18.7	26.2±27.8	17.5±31.9
36-40	7.0±14.9	9.9±13.6	16.8±29.4	17.4±29.2
41-45	11.5±18.1	11.3±20.3	23.8±30.2	22.4±30.9
46-50	15.6±21.8	19.6±25.4	16.7±25.9	20.5±27.6
PND 22				
1-5	23.5±24.4	23.0±15.8	21.8±25.2	33.8±27.8
6-10	26.9±25.2	22.8±21.7	31.3±23.7	25.0±23.1
11-15	24.8±28.1	21.0±22.2	30.1±30.6	32.1±34.2
16-20	27.0±27.4	29.4±23.9	32.8±28.4	28.9±34.4
21-25	26.0±30.7	33.7±31.7	34.3±29.0	34.2±26.6
26-30	27.3±25.9	26.9±28.4	35.4±25.8	42.0±27.2
31-35	30.3±28.0	7.0±11.5	36.6±32.4	36.2±33.9
36-40	23.7±25.7	14.5±15.6	23.4±30.8	29.8±28.1
41-45	27.4±29.5	31.0±28.5	32.3±36.4	41.8±36.2
46-50	28.4±31.5	31.2±33.2	48.4±31.3	45.8±28.5

Interval (min)	Dose (mg/kg)			
	0	0.4	2	10
	PND 60			
1-5	67.5±8.8	67.2±7.3	72.2±8.0	70.1±8.0
6-10	73.2±10.5	69.3±12.6	70.4±13.1	70.8±14.2
11-15	66.7±15.8	64.8±13.4	66.6±11.5	61.4±17.8
16-20	60.9±19.7	55.2±21.9	62.1±13.0	58.1±18.4
21-25	55.8±21.8	61.6±9.1	60.4±16.9	58.8±15.5
26-30	53.5±22.4	64.2±15.8	56.5±25.2	57.3±23.7
31-35	54.3±20.4	55.7±16.1	54.8±18.1	53.3±14.0
36-40	50.9±24.2	51.0±18.3	59.7±18.4	56.4±15.1
41-45	50.1±29.6	51.0±17.6	59.6±26.4	52.1±15.7
46-50	55.0±21.4	60.3±14.0	58.8±20.9	59.3±13.1

a Data were obtained from Table 19 on pages 127-133 in MRID 46153102; n = 10-13. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

** Statistically different from controls at $p \leq 0.01$

28

c) **Auditory startle reflex habituation** - No treatment-related differences were observed in peak amplitude (Table 8a) or latency (Table 8b) of auditory startle response in females. In males, a consistent decrease (not statistically significant, high variability) in mean auditory startle reflex peak amplitude was noted at the high dose (10 mg/kg/day) at PND 23 (↓13-26%) and PND 61 (↓10-31%) that may be attributable to treatment. No treatment-related differences were observed in latency of auditory response in males. Data, recorded for the five blocks of 10 repetitions/block, indicated that habituation patterns were normal. The minor differences ($p \leq 0.05$) in peak amplitude and latency noted in the 0.4 mg/kg males and females were not dose related.

Table 8a. Mean (± SD) auditory startle reflex peak amplitude (V) [a]

Post-natal Day	Repetition	Dose (mg/kg/day)			
		0	0.4	2	10
Males					
PND 23	1-10	377.9±140.2	324.1±113.5	310.6±160.9	280.6±113.0 (↓26%)
	11-20	258.2±50.8	240.7±85.7	257.6±73.9	225.3±52.9 (↓13%)
	21-30	234.9±60.0	207.7±77.7	243.0±67.1	196.2±37.7 (↓16%)
	31-40	209.1±49.0	227.5±65.5	232.4±61.9	197.6±55.4 (↓5%)
	41-50	215.6±52.7	213.7±74.1	208.4±44.9	179.0±37.9 (↓17%)
PND 61	1-10	960.1±254.2	1032.6±285.0	983.6±259.9	857.3±248.3 (↓11%)
	11-20	764.9±255.9	919.7±368.4	826.1±157.1	589.1±205.9 (↓23%)
	21-30	708.9±270.0	822.8±341.2	773.6±256.9	490.4±188.7 (↓31%)
	31-40	645.1±315.5	812.2±236.2	668.9±259.7	579.1±239.5 (↓10%)
	41-50	679.8±326.2	659.3±225.2	584.5±278.7	480.6±164.6 (↓29%)
Females					
PND 23	1-10	286.2±76.2	295.4±91.8	294.9±97.8	349.1±95.2
	11-20	228.4±48.7	261.5±68.0	249.5±73.0	253.7±40.8
	21-30	216.4±41.5	236.0±45.0	246.4±85.9	232.9±27.3
	31-40	214.6±50.7	229.1±63.2	201.9±56.4	198.8±34.2
	41-50	203.8±62.3	227.1±66.8	190.1±50.5	226.7±41.7
PND 61	1-10	658.5±245.0	755.0±262.9	652.4±240.7	703.8±169.7
	11-20	520.9±168.3	731.1±235.7* (↑40)	627.5±245.2	654.6±159.0
	21-30	465.0±91.0	690.1±269.3** (↑48)	526.2±179.5	564.8±191.2
	31-40	460.3±146.4	566.3±231.1	502.8±197.4	513.3±207.7
	41-50	401.2±206.7	506.1±173.0	442.2±188.9	465.7±138.9

a Data were obtained from Table 20 on pages 134-137 in MRID 46153102; n=10-12. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

** Statistically different from controls at $p \leq 0.01$

23

Table 8b. Mean (± SD) time to peak amplitude (ms) [a]

Post-natal Day	Repetition	Dose (mg/kg)			
		0	0.4	2	10
Males					
PND 23	1-10	26.1±5.4	26.9±8.0	25.6±7.5	23.4±5.7
	11-20	20.6±2.7	23.9±8.0	22.7±3.7	21.2±3.5
	21-30	20.6±2.3	23.0±3.9	22.4±4.7	21.1±2.4
	31-40	22.5±2.5	23.6±8.9	21.1±3.7	21.5±3.9
	41-50	20.9±2.8	22.9±9.8	21.5±4.4	21.3±3.3
PND 61	1-10	26.1±4.0	24.5±4.2	26.7±7.0	25.2±4.4
	11-20	24.3±3.3	23.4±3.4	24.0±4.3	24.2±3.1
	21-30	24.9±2.8	24.3±3.9	24.6±3.8	24.8±2.9
	31-40	24.8±2.8	24.3±3.3	24.2±2.9	25.2±3.2
	41-50	27.6±4.3	23.5±2.7*(115)	26.6±6.6	25.2±3.2
Females					
PND 23	1-10	22.9±3.8	23.7±3.3	22.6±4.1	23.0±3.1
	11-20	20.1±2.0	20.3±1.8	20.5±2.9	21.2±2.8
	21-30	21.1±3.0	20.2±1.8	20.0±2.3	20.4±1.9
	31-40	19.8±1.4	19.5±1.3	21.6±3.9	21.3±2.7
	41-50	20.6±2.1	20.7±1.9	20.5±2.2	20.4±2.0
PND 61	1-10	26.1±3.0	23.6±2.5	25.7±4.2	24.8±1.9
	11-20	26.4±4.8	22.9±2.4* (113)	25.0±4.3	24.7±2.9
	21-30	24.6±4.4	24.4±2.5	25.0±5.7	23.8±1.7
	31-40	25.5±4.5	23.5±2.8	27.2±10.0	24.6±2.0
	41-50	28.3±5.0	24.5±3.5	25.4±8.1	24.5±2.1

a Data were obtained from Table 21 on pages 138-141 in MRID 46153102; n=10-12. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

d) Learning and memory testing - No treatment-related differences were observed in the water maze tests at either time point (Tables 9a and 9b). Swimming time in the Y-maze was sporadically increased or decreased during several trials in the males at all dose groups and in the 0.4 and 10 mg/kg females. However, these differences were transient and unrelated to dose. Swimming time in the straight channel was unaffected by the test material. The differences ($p \leq 0.05$) noted in the percent of successful swimming trials in both sexes at either time point were not dose-dependent and/or adverse (Table 9c). Numbers of trials to criterion, errors per trial, and animals that failed to learn were not reported.

Table 9a. Mean (± SD) swimming times (s) in water maze around weaning [a]

Session/Parameter		Dose (mg/kg)			
		0	0.4	2	10
Males					
Learning (PND 24)	Straight channel	4.17±2.26	3.98±2.38	5.16±3.62	4.07±2.40
	Trial 1	14.35±7.26	15.10±6.71	14.33±7.44	12.67±5.69
	Trial 2	10.32±5.68	7.76±4.21	7.90±5.19	9.44±4.93
	Trial 3	10.19±5.99	6.73±3.95* (↓34)	8.40±5.25	5.23±2.98** (↓49)
	Trial 4	7.87±4.65	5.65±3.72* (↓28)	5.42±3.44* (↓31)	4.66±2.78** (↓41)
	Trial 5	7.16±5.72	6.49±4.62	4.77±2.04	6.19±4.83
	Trial 6	5.48±2.63	7.20±3.61	5.95±5.84	4.77±2.52
Memory (PND 27)	Straight channel	2.36±1.07	2.72±1.11	2.55±1.12	3.22±2.46
	Trial 1	5.71±2.96	7.02±3.20	5.47±3.05	7.33±6.30
	Trial 2	5.90±3.43	5.17±4.43	3.12±1.04** (↓47)	4.35±3.02
	Trial 3	3.48±1.83	3.60±1.75	4.17±3.81	5.24±4.22
	Trial 4	3.21±1.63	3.14±0.96	3.48±1.92	4.42±3.68
	Trial 5	3.81±2.65	3.84±2.36	3.96±2.79	4.80±3.47
	Trial 6	3.09±1.49	4.05±2.56	3.95±2.34	4.61±4.09
Females					
Learning (PND 24)	Straight channel	3.82±2.26	4.16±2.56	4.74±4.01	3.95±3.26
	Trial 1	15.17±5.82	15.21±7.63	15.23±7.16	12.89±7.01
	Trial 2	8.88±6.06	8.14±5.79	8.05±5.65	7.91±4.73
	Trial 3	7.48±4.99	6.95±3.21	7.16±5.44	8.08±6.33
	Trial 4	6.54±4.29	6.80±4.12	6.20±3.36	6.29±4.37
	Trial 5	5.95±3.89	4.66±3.04	4.81±2.63	4.24±1.83* (↓29)
	Trial 6	4.24±1.90	5.66±3.23	5.71±4.08	6.75±4.43* (↑59)
Memory (PND 27)	Straight channel	2.71±1.21	2.43±0.62	2.63±1.24	2.88±1.40
	Trial 1	6.94±5.55	5.31±2.69	5.56±2.75	7.58±4.10
	Trial 2	4.42±3.36	5.82±3.11	4.30±2.79	5.58±3.89
	Trial 3	4.43±3.32	5.40±3.69	3.68±2.35	4.55±2.80
	Trial 4	3.54±1.62	4.92±3.68	3.01±1.47	3.87±2.75
	Trial 5	3.49±2.31	4.85±2.74	4.86±3.73	4.34±2.59
	Trial 6	3.81±2.52	5.71±5.36	3.28±2.11	3.80±1.81

a Data were obtained from Table 22 on page 142-145 in MRID 46153102; n=19-24. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

** Statistically different from controls at $p \leq 0.01$

Table 9b. Mean (± SD) swimming times (s) in water maze around PND 60 [a]

Session/Parameter		Dose (mg/kg)			
		0	0.4	2	10
Males					
Learning (PND 59)	Straight channel	3.87±1.97	3.82±1.38	4.06±1.87	3.79±1.19
	Trial 1	11.57±5.66	11.60±4.73	10.05±2.94	11.97±4.73
	Trial 2	7.05±4.87	6.30±2.46	6.49±3.75	5.51±2.07
	Trial 3	5.45±3.07	5.15±3.00	5.11±2.60	5.51±2.32
	Trial 4	5.14±2.40	5.42±2.47	4.72±2.60	5.03±2.25
	Trial 5	5.09±3.52	6.29±4.27	5.65±3.57	4.72±2.01
	Trial 6	7.21±6.95	6.32±4.83	6.30±4.31	6.00±5.93
Memory (PND 62)	Straight channel	3.83±2.82	3.64±1.77	3.82±2.69	3.82±2.19
	Trial 1	4.78±2.23	5.51±4.40	3.89±1.70	4.70±2.09
	Trial 2	7.12±8.21	4.78±3.83	4.55±3.44	5.23±4.98
	Trial 3	7.10±5.06	6.87±4.95	7.62±6.73	6.29±5.84
	Trial 4	6.59±5.64	5.97±3.97	6.23±6.80	8.21±6.17
	Trial 5	7.77±7.05	7.02±5.32	5.77±4.19	6.58±3.81
	Trial 6	7.55±6.39	5.48±3.33	5.64±4.44	5.69±3.49
Females					
Learning (PND 59)	Straight channel	4.06±2.04	3.59±1.04	3.74±1.31	4.37±1.70
	Trial 1	10.93±2.95	12.87±6.13	13.43±5.92	11.90±4.97
	Trial 2	7.05±4.09	7.13±4.17	6.82±3.53	8.63±5.64
	Trial 3	6.04±4.74	5.66±2.27	6.70±4.11	6.03±3.35
	Trial 4	5.58±4.09	5.18±2.95	6.71±4.54	5.21±2.58
	Trial 5	5.26±3.24	4.98±2.07	5.31±3.15	5.21±2.98
	Trial 6	6.14±3.84	6.08±4.25	8.10±5.98	5.34±2.84
Memory (PND 62)	Straight channel	3.80±2.76	3.53±2.74	4.35±4.53	2.73±1.64
	Trial 1	4.63±2.66	6.01±4.66	5.06±3.24	3.95±1.78
	Trial 2	3.53±3.46	6.58±8.06* (↑86)	4.45±4.14	3.64±2.32
	Trial 3	7.88±8.10	8.10±8.79	10.14±8.53	7.38±7.97
	Trial 4	9.07±9.11	11.16±11.30	8.95±7.81	7.36±7.94
	Trial 5	11.08±9.53	8.25±6.98	13.56±10.88	7.99±7.57
	Trial 6	8.79±9.13	10.99±9.33	10.81±9.43	7.85±6.39

a Data were obtained from Table 22 on page 146-149 in MRID 46153102; n=22-24. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

Table 9c. Mean (± SD) percent of successful swimming trials in water maze [a]

Session	Criterion	Dose (mg/kg)			
		0	0.4	2	10
Males					
Learning (PND 24)	≤ 10 seconds	65.9±25.4	69.0±20.6	77.5±13.9	72.9±15.4
	≤ 2 x straight channel time	49.3±33.5	54.0±35.3	64.5±29.0	61.1±28.5
Memory (PND 27)	≤ 10 seconds	94.9±7.8	93.7±9.8	96.4±7.0	88.9±16.8
	≤ 2 x straight channel time	68.8±19.7	69.0±22.5	73.2±28.8	71.5±21.7
Learning (PND 59)	≤ 10 seconds	85.5±19.0	81.8±11.4	85.4±14.2	84.1±15.8
	≤ 2 x straight channel time	69.6±22.3	72.0±18.1	74.3±17.0	69.7±22.2
Memory (PND 62)	≤ 10 seconds	81.2±22.1	84.1±13.4	88.2±15.1	84.1±15.8
	≤ 2 x straight channel time	68.8±30.3	70.6±22.3	73.6±23.0	71.2±25.3
Females					
Learning (PND 24)	≤ 10 seconds	74.2±14.3	74.6±16.1	75.4±15.8	75.7±17.0
	≤ 2 x straight channel time	53.8±27.2	57.9±26.3	60.1±28.3	49.3±29.7
Memory (PND 27)	≤ 10 seconds	93.9±9.7	87.7±17.4	94.2±8.1	91.0±11.0
	≤ 2 x straight channel time	72.7±23.3	63.2±31.7	71.0±20.9	65.3±24.0
Learning (PND 59)	≤ 10 seconds	84.1±13.7	82.6±13.1	72.0±18.1* (↓14)	78.3±17.0
	≤ 2 x straight channel time	71.0±23.1	63.6±22.8	59.1±25.6	70.3±27.5
Memory (PND 62)	≤ 10 seconds	75.4±24.0	71.2±26.3	67.4±27.0	83.3±18.8
	≤ 2 x straight channel time	62.3±22.6	57.6±27.1	59.1±29.9	59.4±25.0

a Data were obtained from Table 23 on page 150-165 in MRID 46153102; n=19-24. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

5. Postmortem results

a) Brain weights - There were no treatment-related effects on brain or cerebellum weights (Table 10). On PND 12, absolute brain and cerebellum weights were decreased in the 0.4 mg/kg males (↓3-6%; $p \leq 0.05$); this decrease, however, was not considered to be treatment related due to lack of time and dose-response. Absolute brain weights were decreased in the 10 mg/kg females (↓6%; $p \leq 0.05$).

27

Table 10. Mean (±SD) brain weights in F_1 rats [a]

Post-natal Day	Parameter		Dose (mg/kg)			
			0	0.4	2	10
Males						
PND 12	**Terminal body weight (g)**		24.1±1.4	23.4±2.5	23.9±1.8	24.3±1.7
	Brain	absolute (g)	1.12±0.03	1.09±0.04* (↓3)	1.11±0.05	1.12±0.04
	Cerebellum	absolute (g)	0.109±0.006	0.103±0.007*(↓6)	0.110±0.008	0.110±0.006
PND 63	**Terminal body weight (g)**		351.9±24.3	352.3±29.0	348.6±19.5	353.4±18.9
	Brain	absolute (g)	1.96±0.08	1.96±0.04	1.97±0.05	1.99±0.09
	Cerebellum	absolute (g)	0.287±0.016	0.289±0.010	0.291±0.023	0.296±0.017
Females						
PND 12	**Terminal body weight (g)**		23.7±2.2	24.3±2.1	23.4±2.4	22.2±2.5
	Brain	absolute (g)	1.08±0.06	1.09±0.04	1.06±0.04	1.02±0.07* (↓6)
	Cerebellum	absolute (g)	0.103±0.010	0.105±0.007	0.099±0.009	0.099±0.012
PND 63	**Terminal body weight (g)**		233.0±17.5	227.3±22.2	223.4±19.7	215.2±11.8
	Brain	absolute (g)	1.87±0.03	1.84±0.07	1.83±0.04	1.83±0.08
	Cerebellum	absolute (g)	0.273±0.015	0.267±0.013	0.262±0.016	0.272±0.015

a Data obtained from Tables 24 and 25 on pages 166-171 in MRID 46153102; n=9-13. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

b) Neuropathology

1) Macroscopic examination -Examination of the uterus revealed that the one control female that did not litter was not pregnant. Increased incidence of minimal eosinophilic infiltrates in the midbrain (thalamus) was noted in the 10 mg/kg females (3/10 treated vs 0/10 controls) on PND 63 (Table 11). It was stated that eosinophil infiltration in the thalamus has been observed as a rare spontaneous finding in developmental neurotoxicity studies using this strain of rat; one control 63 day old male was affected (CTL study number RR0886), and this incidence was only slightly higher than historical controls (data not provided). Although the study author considered this finding to be incidental to naled, since the historical control data were not provided, the significance of this finding is unknown.

28

Table 11. Incidence (# affected/10) of minimal eosinophilic infiltrates in the midbrain (thalamus) in F_1 rats on PND 63. [a]

Dose (mg/kg)			
0	0.4	2	10
Males			
0	NE	NE	0
Females			
0	0	0	3

a Data obtained from Table 30 on pages 192-193 in MRID 46153102.
NE Not examined

2) **Microscopic examination** - There were no treatment-related findings in males or females at PND 12 or 63.

At 10 mg/kg, several brain morphometric measurements were significantly different ($p \leq 0.05$) from the concurrent controls (Tables 12a and 12b): (i) increased thickness of the dorsal cortex in the males on PND 12 (↑5%); (ii) increased width of the dentate gyrus and overall width in the hippocampus in the males on PND 12 (↑6-9%); (iii) decreased length of the dentate gyrus in the hippocampus in the females on PND 12 (↓15%); (iv) increased length from the midline and length of the dentate gyrus in the hippocampus in the males on PND 63 (↑9-11%); and (v) increased width of the dentate gyrus in the hippocampus in the females on PND 63 (↑8%). The findings in both sexes in the hippocampus may be attributable to treatment.

Due to a variety of statistically significant effects seen in the brain morphometric data both in both males and females, examination of the following brain regions from the mid and low dose groups are requested: dorsal cortex, hippocampus, and prepyramidal fissure of the inner granule layer of cerebellum.

Table 12a. Mean (±SD) morphometric measurements in F_1 male rats (mm)[a]

Region/Section	0 mg/kg/day	10 mg/kg/day	0 mg/kg/day	10 mg/kg/day
	PND 12 (N= 8-10)		PND 63 (N=9-10)	
Frontal Cortex:				
Height - Level 2	5.97±0.30	5.81±0.25	6.95±0.51	7.10±0.51
Width - Level 2	4.73±0.26	4.65±0.18	5.06±0.26	5.23±0.40
Dorsal Cortex:				
Thickness (1) - Level 3	1.34±0.07	1.39±0.08	1.37±0.17	1.39±0.12
Thickness (2) - Level 3	1.43±0.07	1.49±0.10	1.74±0.21	1.77±0.15
Thickness - Level 4	1.24±0.06	1.30±0.04*(↑5%)	1.53±0.15	1.49±0.14
Thickness - Level 5	1.15±0.08	1.13±0.06	1.41±0.09	1.35±0.10
Piriform Cortex:				
Thickness - Level 3	1.07±1.10	1.06±0.09	1.29±0.11	1.30±0.12
Thickness - Level 4	1.05±0.07	1.08±0.09	1.27±0.09	1.26±0.10
Thickness - Level 5	1.10±0.10	1.11±0.06	1.17±0.09	1.17±0.10
Hippocampus:				
Length - Level 3	3.29±0.23	3.33±0.36	2.61±0.38	2.46±0.19
Length - Level 4	4.42±0.34	4.53±0.25	3.33±0.39	3.70±0.32* (↑11%)
Overall Width - Level 5	1.32±0.07	1.40±0.10* (↑6%)	1.51±0.12	1.51±0.07
Dentate gyrus length - Level 4	1.47±0.13	1.61±0.25	1.58±0.18	1.73±0.09* (↑9%)
Dentate gyrus width - Level 4	0.52±0.04	0.54±0.03	0.63±0.05	0.64±0.02
Dentate gyrus width - Level 5	0.69±0.06	0.75±0.04* (↑9%)	0.73±0.08	0.75±0.03
Corpus Callosum:				
Thickness - Level 4	0.60±0.10	0.61±0.07	0.31±0.08	0.35±0.07
Thalamus:				
Height -Level 4	5.46±0.21	5.31±0.18	5.08±0.36	5.04±0.48
Width - Level 4	8.18±0.44	8.32±0.22	8.67±0.54	8.67±0.37
Width - Level 5	7.20±0.40	7.36±0.47	7.76±0.57	8.00±0.16
Thalamus/Cortex:				
Overall width - Level 4	13.29±0.60	13.62±0.39	14.57±0.86	14.91±0.37
Cerebellum:				
Height	3.90±0.18	3.83±0.19	5.69±0.34	5.56±0.36
Length	4.45±0.14	4.52±0.25	7.02±0.34	7.10±0.28
Preculminate Fissure Thickness				
Inner granule layer (µm)	143±17	149±18	186±27	164±27
Molecular layer	76.3±9.3	74.5±7.4	219.3±25.7	221.4±16.3
Outer Granular layer (µm)	39.0±7.5	39.0±8.5		
Prepyramidal Fissure Thickness				
Inner granule layer	144±25	142±18	163±26	173±30
Molecular layer	65.6±11.3	62.4±7.8	214.5±27.1	214.3±16.6
Outer Granular layer	45.3±4.1	44.1±6.0		

a Data were obtained from Table 26 on pages 172-188 of MRID 46153102. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Statistically different from controls at $p \leq 0.05$

Table 12b. Mean (±SD) morphometric measurements in F_1 female rats [a]

Region/Section	0 mg/kg/day	10 mg/kg/day	0 mg/kg/day	10 mg/kg/day
	PND 12 (N=8-10)		PND 63 (N=8-10)	
Frontal Cortex:				
Height - Level 2	5.76±0.39	5.54±0.45	6.75±0.28	6.70±0.43
Width - Level 2	4.63±0.29	4.46±0.31	4.95±0.24	4.89±0.31
Dorsal Cortex:				
Thickness (1) - Level 3	1.38±0.10	1.36±0.10	1.35±0.10	1.36±0.10
Thickness (2) - Level 3	1.50±0.07	1.44±0.12	1.73±0.12	1.67±0.12
Thickness - Level 4	1.26±0.09	1.19±0.09	1.43±0.09	1.44±0.15
Thickness - Level 5	1.11±0.08	1.07±0.05	1.34±0.07	1.33±0.08
Piriform Cortex:				
Thickness - Level 3	1.06±0.12	1.06±0.07	1.25±0.11	1.31±0.09
Thickness - Level 4	1.02±0.10	1.02±0.10	1.20±0.07	1.24±0.14
Thickness - Level 5	1.08±0.12	1.08±0.10	1.16±0.08	1.17±0.08
Hippocampus:				
Length - Level 3	3.18±0.18	3.34±0.25	2.48±0.24	2.46±0.20
Length - Level 4	4.51±0.35	4.42±0.34	3.66±0.50	3.64±0.41
Overall Width - Level 5	1.37±0.10	1.35±0.11	1.51±0.03	1.49±0.11
Dentate gyrus length - Level 4	1.66±0.24	1.41±0.20* (↓15%)	1.69±0.21	1.65±0.20
Dentate gyrus width - Level 4	0.54±0.05	0.51±0.06	0.60±0.05	0.65±0.02* (↑8%)
Dentate gyrus width - Level 5	0.72±0.05	0.72±0.07	0.73±0.03	0.73±0.03
Corpus Callosum:				
Thickness - Level 4	0.55±0.11	0.53±0.14	0.30±0.05	0.34±0.07
Thalamus:				
Height -Level 4	5.33±0.18	5.21±0.40	4.48±0.43	5.13±0.37
Width - Level 4	8.28±0.41	8.06±0.61	8.47±0.34	8.58±0.38
Width - Level 5	7.35±0.50	7.02±0.49	7.71±0.29	7.57±0.43
Thalamus/Cortex:				
Overall width - Level 4	13.55±0.90	13.04±0.80	14.57±0.76	14.69±0.78
Cerebellum:				
Height	3.70±0.28	3.64±0.17	5.39±0.21	5.47±0.28
Length	4.36±0.23	4.18±0.27	6.84±0.29	6.76±0.24
Preculminate Fissure Thickness				
Inner granule layer	146±26	145±14	177±31	164±24
Molecular layer	74.5±8.9	77.3±7.3	213.4±17.2	214.2±19.5
Outer Granular layer	39.5±3.6	40.2±5.8		
Prepyramidal Fissure Thickness				
Inner granule layer	149±22	127±29* (↓15%)	146±17	165±25
Molecular layer	62.5±9.3	59.5±8.4	194.5±14	205.8±22.3
Outer Granular layer	43.9±7.4	46.7±8.8		

a Data were obtained from Table 26 on pages 172-188 of MRID 46153102. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Statistically different from controls at $p \leq 0.05$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - It was concluded that Naled administered orally by gavage to pregnant dams from gestation day 7 through lactation day 7, and to the F_1 offspring from post-natal day 8 through 22, produced no adverse effects at up to 10 mg/kg. The maternal and offspring LOAELs were not observed. The maternal and offspring NOAELs were 10 mg/kg. No evidence of developmental neurotoxicity was observed at any dose.

B. REVIEWER COMMENTS

In dams, no treatment-related effects on mortality, clinical signs, FOB, body weight, reproductive performance, or gross pathology parameters were observed at any dose.

Treatment had no adverse effects on offspring survival, clinical signs, FOB, body weight, body weight gain, developmental landmarks, learning and memory, or neuropathology.

A treatment-related decrease in total motor activity (not statistically significant) was seen in F1 males at 2 and 10 mg/kg/day on PND 14 (↓46%, ↓49%, respectively) and PND 18 (↓36%, ↓50%, respectively). [It is noted that the data are highly variable (large standard deviations) for the control and treated groups such that statistical significance was not attained.] The total mean number of movements were increased between PND 14 and 22 in all groups. In the male sub-session data, there was a dose-related decrease in motor activity ($p \leq 0.05$) at 2 mg/kg (↓78%) and 10 mg/kg/day (↓90%) on PND 18 during the 41-45 minute sub-session. These findings are consistent with the overall decrease in motor activity seen on PND 14 and 18 in males at ≥2 mg/kg/day. In addition, the repeated dose cholinesterase studies showed biologically significant decreases in brain cholinesterase activity (↓14-42%) for PND 18 males at the same doses where effects in motor activity were seen in the DNT study.

In males, a consistent decrease (not statistically significant, high variability) in mean auditory startle reflex peak amplitude was noted at the high dose (10 mg/kg/day) at PND 23 (↓13-26%) and PND 61 (↓10-31%) that may be attributable to treatment. No treatment-related differences were observed in latency of auditory response in males. No treatment-related differences were observed in peak amplitude or latency of auditory startle response in females.

Absolute brain weights were statistically significantly decreased (↓6%, $p < 0.05$) in females only at the high dose (10 mg/kg/day). Also at the high dose, several brain morphometric measurements were significantly different ($p \leq 0.05$) from the concurrent controls: (i) increased thickness of the dorsal cortex in the males on PND 12 (↑5%); (ii) increased width of the dentate gyrus and overall width in the hippocampus in the males on PND 12 (↑6-9%); (iii) decreased length of the dentate gyrus in the hippocampus in the females on PND 12 (↓15%); (iv) increased length from the midline and length of the dentate gyrus in the hippocampus in the males on PND 63 (↑9-11%); and (v) increased width of the dentate gyrus in the hippocampus in the females on PND 63 (↑8%).

Due to a variety of statistically significant effects seen in the brain morphometric data both in

both males and females, examination of the following brain regions from the mid and low dose groups are requested: dorsal cortex, hippocampus, and prepyramidal fissure of the inner granule layer of cerebellum.

The maternal NOAEL is 10 mg/kg/day (highest dose tested). The maternal LOAEL is not established.

The offspring LOAEL is 2 mg/kg/day, based on decreased total motor activity in males at PND 14 and 18 and decreased subsession motor activity in males in the 41-45 minute interval on PND 18. The offspring NOAEL is 0.4 mg/kg/day.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6; OECD 426 (draft)) due to the need for additional brain morphometric data as well as pending a comprehensive review of all available positive control data.

Appendix I

Range-finding study for developmental neurotoxicity of Naled in rats

In a range-finding study (MRID 46153101), Naled (95.8% a.i., Batch #JF110299) in dried corn oil was administered via gavage (10 mL/kg) to pregnant Alpk:AP$_f$SD Wistar-derived rats (15/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 3, 10, or 30 mg/kg. Clinical signs, body weight, and food consumption (during gestation only) were recorded for the dams. Five dams/dose were sacrificed on GD 22 and LD 22 for determination of brain and erythrocyte cholinesterase activity using a modified Ellman method. The fetuses were removed from the 5 dams/dose sacrificed on GD 22 and were used for determination of brain and erythrocyte cholinesterase activity. The remaining dams were sacrificed on LD 22 and discarded without examination. The number, sex, survival, clinical condition, and body weights of the pups were monitored. On post-natal days (PND) 2, 8, 15, and 22, 5 pups/sex/dose (1 pup/litter when possible) were sacrificed for determination of brain and erythrocyte cholinesterase activity. No pups were culled to standardize the litter size. Functional observational battery (FOB), motor activity, acoustic startle response, learning and memory, and neuropathology were not examined in the pups.

No adverse treatment-related effects on mortality, clinical signs, food consumption, or gestation length were observed in the maternal animals at any dose. No effects on litter size, number of live born pups, survival, sex distribution, or clinical signs were observed in the pups at any dose.

One 30 mg/kg dam displayed hunched posture, pale, piloerection, pinched in sides, and urine staining prior to dying shortly after giving birth. Clinical signs were limited to staining around the mouth following dosing in 11/15 dams at 30 mg/kg, and 3/15 dams at 10 mg/kg.

At 30 mg/kg, maternal body weights (adjusted for body weight on GD 7) were transiently decreased ($p \leq 0.05$) on GD 8-11 (↓1-2%) and LD 3-15 (↓3-5%). There was no effect on maternal food consumption during gestation. F_1 body weights (adjusted for body weight on PND 1) were transiently decreased ($p \leq 0.05$) in the males (↓8-11%) on PND 5-8, and in the females (↓9-12%) on PND 8-15. Brain cholinesterase activity was decreased ($p \leq 0.05$) by 19-23% in the fetuses of both sexes on GD 22, and by 14% in the male pups on PND 2. Erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) by 23-30% in the fetuses of both sexes on GD 22, and by 35% in the male pups on PND 2.

In the ≥ 10 mg/kg dams, brain cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) by 37-72% on GD 22 and by 66-81% on LD 22. Erythrocyte cholinesterase activity was decreased ($p \leq 0.01$) by 50-53% on GD 22 and by 52-54% on LD 22.

Additionally in the 3 mg/kg dams, erythrocyte cholinesterase activity was decreased ($p \leq 0.01$) by 27% on GD 22 and by 25% on LD 22.

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

34