DATA EVALUATION RECORD

ACEPHATE

Study Type (§83-6a): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 1-01-21 (MRID 46151802)

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DATA EVALUATION RECORD

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

PC CODE: 103301 **DP BARCODE:** D298039
TXR#: 0052318 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Acephate Technical (99.2% a.i.)

SYNONYMS: *O,S*-dimethyl acetylphosphoramidothioate

CITATION: Hoberman, A.M. (2003) Oral (gavage) developmental neurotoxicity study of acephate technical in rats. Argus Research, Horsham, PA. Laboratory Project Id.: VP-23747, December 4, 2003. MRID 46151802. Unpublished.

Hoberman, A.M. (2003) Positive control data to support the study "Oral (gavage) developmental neurotoxicity study of acephate technical in rats." Argus Research, Horsham, PA. Project No. VP-23747, Appendix G. December 4, 2003. 2183 p. MRID 46179301.

SPONSOR: Valent U.S.A. Corporation, 1600 Riviera Avenue, Suite 200, Walnut Creek, CA.

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRID 46151802) Acephate technical (99.2% a.i.; Lot #: AS 40s) in deionized water was administered daily by oral gavage to pregnant Crl:CD® (SD)IGS BR VAF/Plus® rats (25/dose) at doses of 0, 0.5, 1, or 10 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 6. Additionally the F_1 pups were similarly dosed on postnatal days (PNDs) 7-21. Dams were allowed to deliver naturally and were sacrificed on LD 6. On PND 4, litters were standardized to 10 pups/litter (5 males and 5 females when possible); the remaining offspring were sacrificed and examined grossly and for cholinesterase activity. Subsequently, 10 pups/sex/group were allocated to Subsets 1-4 and up to 10 pups/sex/group to Subset 5. Selected subsets were examined for detailed clinical and functional observational battery, motor activity, auditory startle habituation, passive avoidance and water maze learning and memory tests, brain weight, neuropathology, and/or brain and blood cholinesterase determinations. Pups were weaned on PND 21, and all offspring were sacrificed by PND 71.

No treatment-related effect was observed on maternal mortality, clinical signs, abbreviated functional observations, body weight, food consumption, reproductive performance, and gross pathology.

The maternal NOAEL is 10 mg/kg/day (HDT). A maternal LOAEL is not established.

Treatment had no adverse effects on offspring survival, body weight, body weight gain, food consumption, clinical signs, FOB, developmental landmarks, auditory startle reflex, learning and memory, brain weights, brain morphology or neuropathology. Assessment for motor activity revealed a non-significant but dose-related decrease in the number of movements (19% ↓at 1 mg/kg/day to 30%↓at 10 mg/kg/day) that was accompanied by non-significant but comparable dose-related decreases in time spent in movement (19% ↓at 1 mg/kg/day to 28%↓at 10 mg/kg/day) in females on Day 21. However, it was determined that no conclusions can be drawn regarding the effect of acephate on motor activity because the variability in the data was so high.

No treatment-related cholinesterase inhibition (ChEI) was seen in the brains, plasma or red blood cells of male or female pups at PND 4. On Day 21, dose-depended and statistically significant ChEI of the brain were seen. Inhibition at the low, mid and high dose groups were 29 %, 34% and 62%, respectively, in males and 25%, 25%, and 58%, respectively, in females. There were also significant ($p<0.01$) reductions in plasma (46% in males and 43% in female) and RBC (50% in males and 63% in females) ChEI in males at the high-dose males at PND 21.

The offspring LOAEL is 0.5 mg/kg/day (LDT), based on statistically significant and dose-depended inhibition of brain cholinesterase activity in male and female pups on Day 21. An offspring NOAEL was not established.

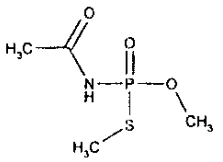
This study is classified **Acceptable/NonGuideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of motor activity in the offspring and the pending comprehensive review of the positive control data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Acephate technical

Description:	White powder
Lot #:	AS 40s
Purity:	99.2% a.i.
Compound Stability:	Not provided.
CAS # of TGAI:	30560-19-1
Structure:	

2. Vehicle - Reverse osmosis process water (gavage treatment)

3. Test animals (P)

Species:	Rat	
Strain:	Crl:CD® (SD)IGS BR VAF/Plus®	
Age at study initiation:	Approximately 68-71 days	
Weight study initiation:	217-242 g (females)	
Source:	Charles River Laboratories, Inc., Portage, Michigan	
Housing:	Individually in stainless steel, wire bottom cages, until GD 20 then individually in nesting boxes; each dam and delivered litter was housed in a common nesting box during the postpartum period; after PND 20, offspring were housed in stainless steel, wire bottomed cages	
Diet:	Certified Rodent Diet® #5002 (PMI Nutrition International, Inc., St. Louis, MO), *ad libitum*	
Water:	Tap water processed by reverse osmosis and then chlorinated, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	≥10/hr
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	6-9 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 10/28/02 End: 11/20/02

2. Study schedule - The maternal animals were mated and assigned to study. The P females were administered the test substance once daily via oral gavage from gestation day (GD) 6 until lactation day (LD) 6. On postnatal day (PND) 4, litters were standardized to 10 pups each (5 male and 5 female pups, when possible). The P females that did not deliver a litter were sacrificed on GD 25. Dams and pups not selected for continued observation on PND 4 were sacrificed. All remaining dams were sacrificed on LD 21 (weaning). The remaining pups were sacrificed on PND 70 or 71.

3. Mating procedure - After acclimation, females were paired (1:1) with males of the same strain and source. The cohabitation period lasted a maximum of five days and was discontinued when

successful mating was verified by the presence of a copulatory plug or sperm in a vaginal smear. The day of successful mating was designated as GD 0, and the females were assigned to individual cages.

4. Animal assignment - Mated females were randomly assigned (stratified by body weight) to test groups as shown in Table 1. Offspring were standardized to 10 pups/litter at PND 4 and assigned to 5 subsets. Each subset contained (when possible) 5 pups/sex with one male and one female/litter/dose group. Cholinesterase activity was determined from blood and brain samples collected from 10 pups/sex/dose from animals not assigned to a subset and/or Subset 5 on PND 4 and from pups in Subset 1 that were not selected for neurohistological examination on PND 21. Animals in Subset 5 were used to replace any dead animals of the other subsets. In the learning and memory tests, the same animals were subjected to two different types of tests. The same individual animals assigned to FOB and motor activity testing were evaluated at all schedule time points.

Table 1. Study design. [a]

Experimental Parameter	Dose (mg/kg/day) 0	0.5	1	10	Subset
Maternal Animals					
No. of maternal animals assigned	25	25	25	25	--
Offspring					
Detailed clinical/FOB (PNDs 4, 11, 21, 35, 45, and 60)	20/sex	20/sex	20/sex	20/sex	4
Motor activity (PNDs 13, 17, 21, and 58)	20/sex	20/sex	20/sex	20/sex	3
Auditory startle habituation (PNDs 22 and 62)	20/sex	20/sex	20/sex	20/sex	3
Learning and memory Passive avoidance (PNDs 22-24 and 29-31) & Water maze (PNDs 58-62 and 65-69)	20/sex	20/sex	20/sex	20/sex	2
Brain weight PND 21 PND 71	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 1 4
Neuropathology PND 22 PND 70	 10/sex 10/sex	 0/sex 0/sex	 0/sex 0/sex	 10/sex 10/sex	 1 4
Brain and blood cholinesterase determination PND 4 PND 21	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 -- 1

a Data were obtained from MRID 46151802 (pages 27-28 and 708-715).

5. Dose-selection rationale - The Sponsor stated that doses were selected based on the results of a dose rangefinding developmental neurotoxicity study in rats (Argus Research Study 222-002P; VP-23739), and rangefinding studies in neonatal rats (Argus Research Study 222-003; VP-25056) and adult rats (Argus Research Study 222-004; VP-25064). Further details were not provided.

6. Dosage administration - All doses were administered once daily to maternal animals by gavage, on GD 6 through LD 6, in a volume of 10 mL/kg of body weight/day. The volume was adjusted daily based on the most recent body weight determination. Dams in the process of delivering pups were not intubated, but no dam missed more than one daily intubation. The F_1 pups were similarly dosed once daily by gavage on PNDs 7-21.

7. Dosage preparation and analysis - Formulations were prepared weekly by mixing appropriate amounts of test substance with reverse osmosis membrane processed deionized water and were stored at 2-8°C. Homogeneity and stability testing were not conducted in this study. It was stated that the Sponsor can document the solubility of the test substance in the vehicle for the tested dose range and has stability data for prepared formulations bracketing the range of doses tested in this study; however, these data were not provided to the reviewers. Concentration analyses were performed on duplicate samples collected from each dose on the first and last days of preparation.

Results - **Concentration Analysis** (range as % of nominal): 96.1-99.9

Assuming that the formulations are stable and homogeneous, the analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals - The dams were checked for mortality twice daily. Clinical observations of the dams were conducted during acclimation, on GD 0, during parturition, and once daily on GDs 8-25 and LDs 0-21. The Sponsor stated that clinical observations were conducted on a schedule that the Study Director deemed appropriate; however, further information was not provided. The results show no clinical observations were reported for GDs 1-7; it is unknown if clinical observations were made during this period.

All dams were observed daily outside the home cage before dosing and on GD 6 through LD 6 at approximately the same time each day by an investigator who was unaware of each rat's dose group. It was not stated if the same technicians observed the animals throughout testing. The following abbreviated functional observations were reported, but without severity data:

ABBREVIATED FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic dysfunction, including: 1) Lacrimation and salivation 2) Piloerection and exophthalmus, 3) Urination and defecation 4) Palpebral closure 5) Respiration 6) Prominence of the eye
X	Abnormal movements
X	Abnormal postures
X	Abnormal behaviors and unusual appearance

Further details concerning the abbreviated functional observations and the maternal animals were not provided.

Individual maternal body weights and food consumption were measured during the acclimation period (body weight only), on GD 0, daily during the dosing period, on LDs 7, 11, 14, 17, and 21, and at sacrifice (body weight only).

Animals were examined for duration of gestation, litter sizes, live litter size, and pup viability at birth. Maternal behavior was evaluated on LDs 0, 4, 7, 11, 14, 17, and 21.

b. Offspring

1) Litter observations - The day of completion of parturition was designated as PND 0. Each litter was evaluated for viability at least twice daily, and live pups were counted once daily. The date that the pups were sexed was not provided, but occurred prior to standardization on PND 4. Pups were weighed individually on PND 0 and 4 (before and after standardization), and daily from PNDs 7-21 (treatment period). Clinical observations were performed once daily during pre-dosage period, daily before administration during dosage period, and weekly during post-dosage period.

On PND 4, litters were standardized from 20 randomly selected litters/dose group of the appropriate size. The litters were reduced to 10 pups each with 5 pups/sex when possible. Whole blood and brain samples were taken for cholinesterase assays from animals not chosen for continued observation at PND 4.

2) Developmental landmarks - Beginning on PND 38, male offspring in Subsets 2-4 were examined daily for preputial separation. Beginning on PND 27, female offspring in Subsets 2-4 were examined daily for vaginal patency. The age of onset was recorded, and body weights were recorded for each rat on the day of sexual maturation.

3) Postweaning observations - After weaning on PND 21, live pups were counted once daily. Each week, pups were weighed, food consumption was recorded, and clinical observations were conducted. Body weights were also measured on the day of sexual maturation and at sacrifice.

4) Neurobehavioral evaluations - Observations and the schedule for those observations are summarized as follows from the report.

i) Abbreviated functional observations - On PNDs 4, 11, 21, 35, 45, and 60, the Subset 4 animals (20 pups/sex/dose) were examined outside of the home cage. The same parameters assessed in the maternal abbreviated functional observations were examined in the offspring by an individual who was unaware of each rat's dose group. Additional information was not provided.

ii) Motor activity testing - Motor activity measurements were performed on 20 offspring/sex/dose (Subset 3) on PNDs 13, 17, 21, and 58 before dosing. Movement was monitored by a passive infrared sensor (make and source not provided) mounted outside a stainless steel, wire-bottom cage. Plexiglass® flooring was used before PND 21. Data were collected in ten-minute intervals over the course of 60 minutes. Number of movements and time spent in movement was recorded. Each rat was tested in the same location on the rack across test sessions. Groups were counterbalanced across testing sessions and cages.

iii) Auditory startle reflex habituation - Auditory startle response and habituation testing was performed on 20 offspring/sex/dose (Subset 3) on PNDs 22 and 62 using an automated system (make and source not provided). The Sponsor provided the following details. The rats were tested in sets of 4 within a sound-attenuated chamber. Each rat was placed inside a small cage situated above a platform containing a force transducer in its base. A microcomputer sampled the output of the force transducer and controlled the test session. The rats initially underwent an adaptation period of 5 minutes. During the last minute of this period, 10 "blank" trials were conducted to sample the baseline force in the absence of stimulus. The rats were then presented with 30 msec, 120 dB bursts of noise at 10-second intervals for 50 trials. An additional 10 "blank" trials followed. The peak amplitude of each response was recorded, and the average response on baseline trials subtracted to calculate the response magnitude. The average response magnitude and the pattern of responses over 10 trial blocks were compared among the dose groups.

iv) Learning and memory testing - Learning and memory testing were performed on 20 offspring/sex/dose (Subset 2). Passive avoidance testing was performed on PNDs 22-24 and 29-31. Water maze testing was performed on PNDs 58-62 and 65-69.

The passive avoidance test was meant to evaluate learning, short-term retention, long-term retention, activity, and hyperactivity. The Sponsor provided the following details. The passive avoidance apparatus consisted of a two-compartment chamber with hinged Plexiglas® lids. One compartment was fitted with a bright light and Plexiglass® floor. The other compartment was fitted with a grid floor to which a 1 second pulse of 1 mA current could be delivered. The two compartments were separated by a sliding door. On each test trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore the apparatus until it entered the "dark" compartment. The sliding door was then immediately closed, the light was

turned off and the brief pulse of current was delivered to the grid floor. The rat was then removed from the apparatus and placed into a holding cage for 30 seconds before the start of the next trial. Trials were repeated until the rat remained in the "bright" compartment for 60 seconds on two consecutive trials (the criterion for learning) or until 15 trials had been completed. The latency to enter the dark compartment or the maximum 60-second interval was recorded for each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval, and the criterion was the same for both days of testing. Dose groups were compared for the following dependent measures: the number of trials to the criterion in the first session (this measure was used to compare groups for overall learning performance), the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first session (this measure was used to compare groups for activity levels and exploratory tendencies in a novel environment), the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session (this measure was used to compare groups for short-term retention), the number of trials to the criterion in the second test session (this measure was used to compare groups for long-term retention) and latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session (this value was another indication of long-term retention).

The watermaze test was meant to evaluate overt coordination, swimming ability, motor activity, learning, and memory. The Sponsor provided the following details. Each rat was tested in a watertight, 16-gauge, stainless steel, modified M-maze. The maze was filled with water to a depth of approximately nine inches; the water was monitored for temperature (range of 21°C ± 1°C). On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal and were then removed from the water. A 15-second inter-trial interval separated each trial. Each rat was required to reach a criterion of 5 consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Each rat was tested twice. The test sessions were separated by a 1-week interval; the correct goal and criterion were the same for both test sessions. Dose groups were compared for the following dependent measures: the number of trials to criterion on the first day of testing (this measure was also used to compare groups for overall learning performance), the average number of errors (incorrect turns in the maze) for each trial on the first day of testing (this measure was also used to compare groups for overall learning performance), the latency (in seconds) to reach the correct goal on trial 2 of the first day of testing (this measure was used to compare groups for short-term retention), the number of trials to criterion on the second day of testing (this measure was used to compare groups for long-term retention), the average number of errors on each trial on the second day of testing (this measure was also used to compare groups for long-term retention) and the latency (in seconds) to reach the correct goal on trial 1 of Day 2 of testing (this was another indicator of long-term retention).

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5) Cholinesterase determination - Cholinesterase activity was determined from blood and brain samples collected from 10 pups/sex/dose from animals not assigned to a subset and/or Subset 5 on PND 4 and from pups in Subset 1 that were not selected for neurohistological examination on PND 21. Animals came from 10 litters/dose group. Whole blood samples were collected via cardiac puncture on PND 4 and inferior vena cava on PND 21 at 3 hours post-dose. Blood samples were transferred to tubes containing EDTA and were processed to separate RBC and plasma. Blood samples were pooled by sex and litter and stored at 2-8°C until analysis. The brains were excised, weighed, placed in saline and stored at 2-8°C until analysis.

Samples were analyzed according to the Charles River Argus SOP 33B2.40 method. RBC samples (after washing in saline) and brain samples were extracted with 0.1% Tween® 80, pH 8.0, and diluted as needed. Plasma samples were analyzed directly. All samples were analyzed in duplicate.

2. Postmortem observations

a. Maternal animals - Dams were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Sacrifice occurred on: (i) PND 21; (ii) when the last pup was found dead or missing if before PND 21; (iii) LD 4 for those dams not selected for continued observation; (iv) LD 21 for those dams selected for continued observation; and (v) GD 25 for rats that did not deliver.

b. Offspring - The lungs of pups that died before initial examination of the litter for pup viability were excised and immersed in water. Pups with lungs that sank were considered stillborn; otherwise, the pups were considered to have died shortly after birth.

Pups were culled on PND 4 using an intraperitoneal injection of sodium pentobarbital. These animals were necropsied and examined for external gross lesions, and pups with gross lesions were preserved in Bouin's solution.

Ten offspring/sex/group were assigned for brain weight measurements on PNDs 21 (Subset 1) and 71 (Subset 4). These rats were administered a combination of sodium heparin and sodium pentobarbital and perfused *in situ* with neutral buffered 10% formalin. The rats were examined for gross lesions. The brains, heads, spinal columns, and hindlimbs were excised and shipped in neutral buffered 10% formalin for weighing, morphological measurements, and neurohistology. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed on: (i) 10 pups/sex/group (Subset 1) and the remaining pups of Subset 5 on PND 21; (ii) 10 pups/sex/group on PND 71 (Subset 4); and (iii) on all rats that were found dead.

Brain measurements were performed using Vernier calipers by an individual who was unaware of each rat's dose group. Brain measurements included the length of the cerebrum from the anterior to posterior pole, exclusive of the olfactory bulbs, and a linear measurement of the cerebellum extending from the anterior edge of the cerebellar cortex to the posterior pole.

After brain measurements, slides of brain samples were prepared for histological examination. These sections (embedded in paraffin) included the following: (i) coronal slices through the

cerebrum at the level of optic chiasm, infundibulum, and mammillary bodies; (ii) coronal slice through the middle of the cerebellum; (iii) multiply-embedded sections including the olfactory bulbs, two coronal slices through the anterior pole, one coronal slice through the cerebrum at the level of the midbrain, one through the posterior portion of the cerebellum, and one through the medulla oblongata; (iv) longitudinal sections of the Gasserian ganglia and associated trigeminal nerves; (v) longitudinal sections of the dorsal root ganglia and spinal nerve roots; and (vi) cross and longitudinal sections of the spinal cord. The following tissues were embedded in glycol methacrylate: cross sections of the sciatic and tibial nerves, longitudinal section of the sciatic nerve, and longitudinal sections of the common peroneal (fibular), tibial, and sural nerves. Only the coronal slices and multiply-embedded sections were prepared for the PND 22 rats (Subset 1), while all slices were examined at PND 70 (Subset 4).

The following morphometric measurements were performed: (i) thickness of the frontal cortex, parietal cortex, corpus callosum, and hippocampal gyrus; (ii) diagonal width (maximum cross-sectional width) of the caudate putamen and underlying globus pallidus; (iii) diagonal width of the coronal section taken at the level of the optic chiasm; and (iv) maximum height of the cerebellum. All measurements were taken bilaterally (except the maximum height of the cerebellum) and recorded separately.

Tissues for histological examination were stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and/or with Bielschowsky's technique (a silver stain for axons and neuronal cytoarchitecture). All histology sections from each rat in the control and 10 mg/kg/day groups were microscopically examined. All microscopic findings were graded for severity [1 of 5 severity grades (minimal, mild, moderate, marked or severe)] and given distribution qualifiers (focal, multifocal or diffuse) . Only selected regions were entered into a PC-based data collection system (GLPath™). Thirty-three regions were entered for rats sacrificed at PND 22 , and 42 regions were entered for rats sacrificed at approximately PND 70. These regions included the examination of the following tissues.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN[a]		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic nerve
X	Cerebral cortex		
X	Midbrain		**OTHER**
X	Cerebellum	X	Sural Nerve
X	Hippocampus	X	Tibial Nerve
X	Medulla oblongata	X	Peroneal Nerve
X	Basal forebrain	X	Dorsal root ganglia
X	Thalamus		
X	Hypothalamus		
	SPINAL CORD		
X	Cervical		
X	Lumbar		
X	Thoracic		
	OTHER		
X	Gasserian ganglion		
X	Trigeminal nerves		
	Optic nerve		
	Eyes		
	Skeletal muscle		

a Only regions of the brain were examined in the animals that were sacrifice on PND 22 (Subset 1).

D. DATA ANALYSIS

1. Statistical analyses - The data were tested ($p \leq 0.05$ and ≤ 0.01) using the following statistical methods:

Parameter	Statistical Methods
Interval or ratio data including body weights, food consumption, latency and error per trial scores in behavioral tests, and percent mortality per litter	Bartlett's test. If significant ($p \leq 0.001$), the Kruskal-Wallis test and Dunn's test. Otherwise, ANOVA and Dunnett's test.
Repeated measurements including data from motor activity and auditory startle habituation test	ANOVA with repeated measures. If significant, Dunnett's test, and One-way ANOVA for each block and Dunnett's test
Graded or count score data including litter size, number of trials to a criterion in a behavioral test or the day a developmental landmark appeared	Kruskal-Wallis test and Dunn's test. Fisher's Exact test on proportion of ties when >75% ties at any concentration.
Clinical observations and other proportion data	Variance test for homogeneity of the binomial distribution
Brain weights	Dunnett's T-test
Morphometric parameters	Single factor ANOVA, mean values of bilateral measurement were used for comparison
Histology	Fisher's Exact test

2. Indices

a. Reproductive indices: The following proportions were reported for females: those that were pregnant, delivered litters, had stillborn pups, had no live born pups, those with all pups dying by PND 4, and with all pups dying by PND 21. The gestation index was also reported.

$$\textbf{Gestation index (\%)} = \frac{\text{\# of rats with live offspring} \times 100}{\text{\# of pregnant rats}}$$

b. Offspring viability indices: The proportion of pups that were live born, stillborn, and found dead or presumed cannibalized at PND 0 and PND 1-4 were reported. The viability index was also reported.

$$\textbf{Viability index (\%)} = \frac{\text{\# live pups on PND 4 (pre cull)} \times 100}{\text{\# live born pups on PND 0}}$$

3. Positive control data - Positive control data for neurobehavior and neuropathology were presented in MRID 46179301, and evaluated for proficiency (see Appendix I). Most of the positive control studies are unacceptable for use with the current study. Few of the studies were conducted within the last few years before the current study. The majority of the studies did not utilize immature rats as test subjects. None of the studies that included motor activity assessment used a 1.5-hour session with 5-minute blocks. Few of the studies included complete descriptions of the methods used or tables of individual data. None of the studies demonstrated the laboratory's ability to detect major functional neurotoxic endpoints using the observational methods used in the current study. However, since this laboratory has historically demonstrated some ability to detect neurobehavioral and neuropathological effects in guideline DNT testing, additional positive control data will not be required to accept this study. It is also noted that insufficiently sensitive procedures could lead to a failure to detect effects on some parameters (for example, cognitive or motor activity testing) or a failure to detect effects at low doses.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality, clinical signs, and functional observations - No unscheduled deaths occurred during the study. No treatment-related clinical signs were noted at any dose during gestation or lactation.

2. Body weight and food consumption - Selected group mean body weights and food consumption values for pregnant and nursing dams are presented in Tables 2a and 2b. No treatment-related effect was observed on body weights and body weight gains or on food consumption in the P females. Differences ($p \leq 0.05$) in body weight gain and food consumption were unrelated to dose.

Table 2a. Selected mean (± SD) body weights (g) for P females administered acephate from GD 6 through LD6. [a]

Gestation Day	Dose (mg/kg/day)			
	0	0.5	1	10
Gestation (n=23-25)				
0	228.9±6.7	228.9±7.1	227.7±7.1	228.2±7.0
6	259.6±11.7	259.9±9.0	256.8±9.3	260.0±11.2
13	293.4±18.0	293.0±14.8	290.2±13.5	293.2±16.1
20	363.8±24.6	364.0±22.4	363.2±18.1	360.4±23.0
Gain, Days 0-20	134.9±20.9	135.0±19.6	135.5±14.8	132.3±20.7
Lactation (n=23-24)				
0	281.1±16.4	280.0±15.8	281.8±17.5	279.3±18.9
7	300.4±18.7	303.3±20.5	295.6±15.9	301.0±15.7
14	326.8±20.1	328.2±23.0	324.5±17.9	328.8±22.2
21	315.6±21.2	306.5±25.8	311.8±16.2	320.2±19.6
Gain, Days 0-7	17.9±12.3	24.2±15.4	13.7±13.8	18.8±13.4
Gain, Days 7-21	15.2±15.4	3.2±21.4* (179)	16.2±15.9	19.2±12.5

a Data were obtained from pages 90-93 of MRID 46151802. Percent difference from control (calculated by reviewers) is presented parenthetically.

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

Table 2b. Mean (± SD) cumulative food consumption in P females administered acephate from GD 6 through LD6. [a]

Interval (Days)	Dose (mg/kg/day)			
	0	0.5	1	10
Gestation (n=23-25)				
Absolute overall (Days 0-20, g/animal/day)	21.9±1.6	21.9±1.9	21.6±1.3	21.8±1.7
Relative overall (Days 0-20, g/kg/day)	74.0±3.1	74.2±4.0	73.8±3.2	73.8±3.4
Lactation (n=23-24)				
Absolute (Days 0-7, g/animal/day)	32.6±4.4	37.9±6.2** (↑16)	34.4±5.2	36.7±6.1* (↑13)
Absolute (Days 7-21, g/animal/day)	65.0±7.2	66.7±5.9	63.8±7.8	66.9±6.7
Relative (Days 0-7, g/kg/day)	114.0±11.6	132.1±19.2** (↑16)	120.8±15.6	127.2±20.8* (↑12)
Relative (Days 7-21, g/kg/day)	202.6±18.7	209.7±13.7	201.5±21.7	208.4±16.8

a Data were extracted from pages 94-97 of MRID 46151802. Percent difference from control (calculated by reviewers) is presented parenthetically.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

3. **Reproductive performance** - No treatment-related effect on the reproductive performance was observed (Table 3).

Table 3. Delivery observations in P females administered acephate from GD 6 through LD6. [a]

Observation	Dose (mg/kg/day)			
	0	0.5	1	10
# of females mated	25	25	25	25
# pregnant	24	23	23	25
# of litters	24	23	23	25
Gestation index (%)	100	100	100	96
Means (±SD) gestation duration (days)	22.2±0.5	22.3±0.5	22.4±0.5	22.5±0.6

a Data were obtained from page 98 of MRID 46151802.

4. **Maternal postmortem results** - No treatment-related effect was observed during necropsy of the P females.

B. OFFSPRING

1. **Viability and clinical signs** - No treatment-related effects were observed on the viability (Table 4) and clinical signs of the offspring.

Table 4. F_1 live litter size and viability. [a]

Observation	Dose (mg/kg/day)			
	0	0.5	1	10
Number of litters	24	23	23	25
Total # of pups delivered	302	309	317	322
# of liveborn	299	309	317	319
# of stillborn	2	0	0	3
Sex ratio (% male)	54.8±15.2	47.2±13.7	51.1±13.9	48.1±13.0
Mean live pups/litter (total pups)				
PND 0	13.0±2.0	13.4±2.5	13.8±1.5	13.3±2.5
PND 4 (Pre-culling)	13.0±1.9	13.3±2.5	13.7±1.6	13.2±2.5
PND 4 (Post-culling)	10.2±1.4	10.0±1.2	10.2±0.6	10.0±0.8
# of deaths (PND 0)	0	0	1	1
(PNDs 1-4)	3	2	0	1
(PND 71)[b]	2	1	3	1
Viability index (%)	99.0	99.4	99.7	99.4

a Data were obtained from pages 99 and 100 of MRID 46151802.

b Two males in the 1-mg/kg/day group were found dead on PND 8. The other deaths were in females which were found dead, sacrificed *in extremis*, or presumed cannibalized on PNDs 6-43.

2. <u>Body weight</u> - No treatment-related effect was observed on offspring body weight (Tables 5a and 5b). Differences ($p \leq 0.05$) were observed, but were unrelated to dose.

Table 5a. Selected mean (± SD) F_1 animal pre-weaning body weights and body weight gains (g). [a]

Post-natal Day	Dose (mg/kg/day)			
	0	0.5	1	10
Males				
0[b]	6.3±0.4	6.3±0.4	6.3±0.5	6.2±0.6
4 (Pre-culling)[b]	9.2±1.0	9.8±1.1	9.4±1.0	9.4±1.1
4 (Post-culling)[b]	9.2±1.0	10.0±1.1	9.5±1.0	9.6±1.1
7	13.7±2.7	15.9±2.3** (↑16)	13.7±2.9	14.0±2.9
21	46.4±7.2	49.7±6.3** (↑7)	45.9±7.7	45.7±8.1
Gain, Days 7-21	32.8±5.1	33.9±4.6	32.0±5.3	31.7±5.8
Females				
7	13.2±2.4	15.2±2.2** (↑15)	13.2±2.9	13.6±2.8
21	45.3±6.4	48.5±5.5** (↑7)	44.1±7.6	44.7±7.1
Gain, Days 7-21	32.0±4.6	33.3±3.7	31.0±5.0	31.1±4.9

a Data were obtained from pages 101 and 177-184 of MRID 46151802. Percent difference from controls (calculated by reviewers) is presented parenthetically.

b Pup weight/litter (g) is reported. Data were not reported for separate sexes, except in a subset. In this subset, males were slightly heavier than females at PND 4 (page 104).

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

Table 5b. Selected mean (± SD) F_1 animal post-weaning body weights and body weight gains (g).[a]

Post-natal Day	Dose (mg/kg/day)			
	0	0.5	1	10
Males				
22	48.6±8.4	52.2±7.4* (↑7)	48.6±8.2	48.5±8.7
36	148.8±18.2	157.8±15.0** (↑6)	145.5±18.2	147.5±17.3
50	272.6±24.8	282.8±23.2* (↑4)	262.1±26.1* (↓4)	266.6±25.7
71	413.8±31.0	425.9±33.3	398.1±33.0* (↓4)	409.3±37.5
Gain, Days 22-71	365.3±26.0	373.6±30.6	349.4±27.8** (↓4)	360.8±34
Females				
22	47.6±6.7	51.4±5.4* (↑8)	46.1±9.3	47.2±8.5
36	129.4±13.2	138.8±12.3** (↑7)	125.4±16.4	128.2±15.9
50	189.0±18.0	201.5±19.2** (↑7)	182.8±19.0	189.9±20.6
71	248.6±26.1	263.4±26.0** (↑6)	240.1±24.6	251.0±29.3
Gain, Days 22-71	201.2±24.0	212.0±24.0	194.0±19.2	203.4±25.7

a Data were obtained from pages 177-184 of MRID 46151802. Percent difference from controls (calculated by reviewers) is presented parenthetically.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

3. Developmental landmarks

a. Sexual maturation - No treatment-related effect was observed on sexual maturation (Table 6). A minor decrease ($p \leq 0.01$, 3%) in the time to preputial separation was observed in the 0.5 ppm males, however, the effect was associated with the size of the males (significantly ↑ body weight in this dose group from days 22 to 50) rather than a dose-related effect. An even slighter decease in the time to vaginal patency was also seen at this treatment level and again appeared to be asociated with the significantly increased body weight of the females from days 22 to 71.

Table 6. Sexual maturation (mean days ± SD) in F_1 generation rats.[a]

Parameter	Dose (mg/kg/day)			
	0	0.5	1	10
N (M/F)	60/58	60/59	59/57	60/59
Preputial separation (Males)	46.0±2.5	44.6±2.9** (↓3%)	45.5±2.7	46.3±4.2
Vaginal patency (Females)	33.0±2.2	32.5±2.6	33.4±2.2	33.3±2.5

a Data were obtained from page 189 of MRID 46151802.
** Significantly different from controls at $p \leq 0.01$

b. Physical landmarks - Data were not provided.

4. Behavioral assessments

a. Functional observational battery - No treatment-related effects were observed during the functional observational battery.

b. Motor activity - As shown in Tables 7a and 7b, it was noted that in the maternal animals at PND 21, there was a nonsignificant but dose-related decrease in the number of movements (19% ↓at 1 mg/kg/day to 30%↓at 10 mg/kg/day) that was accompanied by nonsignificant but comparable dose-related decreases in time spent in movement (19% ↓at 1 mg/kg/day to 28%↓at 10 mg/kg/day) at these levels. Although changes appear to be treatment-related (e.g., PND 21 females), there is marked variability in the data. Therefore, no conclusions can be drawn regarding motor activity because of variability in the data. Habituation was unaffected by treatment. The number of movements and time spent in movement were least at PND13 and most at PND 58, and was slightly less at PND 21 than at PND 17.

Table 7a. Mean (±SD) motor activity data (number of movements) in F_1 pups in Subset 3. [a]

Post-natal Day	Dose (mg/kg/day)			
	0	0.5	1	10
Males				
13	310.1±167.6	443.7±162.9	341.0±169.0	297.6±171.2
17	473.1±235.3	537.1±219.6	470.9±218.7	458.9±212.7
21	446.0±204.9	507.2±177.7	437.8±157.1	405.9±201.9
58	756.4±85.8	784.0±83.9	793.4±82.7	755.0±108.7
Females				
13	435.2±195.5	435.8±177.3	361.1±186.3	365.0±173.9
17	612.6±176.4	595.3±166.2	586.7±237.6	551.8±161.1
21	549.9±185.9	527.5±191.8	447.8±179.7 (19% ↓)	386.9±173.9 (30% ↓)
58	884.9±87.4	850.4±83.9	779.1±136.4	823.7±124.1

a Data (n=20) were obtained from pages 435-442 of MRID 46151802.

Table 7b. Mean (±SD) motor activity data (time in seconds spent in movement) in F_1 pups in Subset 3. [a]

Post-natal Day	Dose (mg/kg/day)			
	0	0.5	1	10
Males				
13	345.4±253.9	579.4±298.9	416.0±266.9	373.3±327.3
17	718.6±420.4	822.0±406.3	703.1±423.4	694.7±380.4
21	675.0±358.6	727.4±303.7	633.0±269.4	584.9±320.8
58	1610.5±356.6	1541.9±288.6	1488.4±308.2	1623.6±321.4
Females				
13	510.0±301.9	555.8±300.5	453.4±301.9	424.0±212.5
17	942.6±398.9	935.4±330.9	934.4±468.6	826.5±346.5
21	815.6±345.3	792.7±341.0	662.6±300.6 (19% ↓)	583.6±335.8 (28% ↓)
58	1819.0±284.0	1777.3±196.5	1665.4±306.7	1855.6±294.4

a Data (n=20) were obtained from pages 435-442 of MRID 46151802.

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c. **Auditory startle reflex habituation** - No significant treatment-related effect was observed on the auditory startle reflex (Table 8). However, it was noted that there was minimal habituation in the high-dose weanlings of both sexes. As observed earlier, the extreme variation in these data (for certain data points, the SD was higher than the mean) presumably accounts for the lack of statistical significance. The greatest amplitude of response was observed in Block 1 for all groups.

Table 8. Mean (±SD) auditory startle reflex maximum amplitude (g) data from F_1 rats in Subset 3. [a]

Observation [b] (Response Magnitude) [c]		Dose (mg/kg)			
		0	0.5	1	10
Males					
PND 22	Block 1	21.34±12.63	18.68±14.27	15.67±6.51	17.21±7.39
	Block 2	14.64±9.66	12.41±10.19	11.54±6.70	13.79±7.47
	Block 3	13.38±9.73	10.81±10.15	10.32±6.80	14.21±10.00
	Block 4	13.85±9.36	9.60±9.17	11.21±5.57	15.74±8.46
	Block 5	14.03±10.29	11.33±10.88	10.96±7.57	15.51±9.11
	Average	15.460±8.967	12.560±10.390	11.935±5.162	15.285±7.131
PND 62	Block 1	67.25±41.57	65.89±43.26	45.49±22.32	62.20±47.95
	Block 2	45.32±35.96	37.13±28.72	32.00±20.76	35.12±37.07
	Block 3	39.19±37.72	33.32±32.64	28.16±21.06	29.21±30.23
	Block 4	30.85±24.33	32.34±28.81	21.56±15.22	29.55±26.88
	Block 5	30.16±29.83	26.98±24.24	24.85±15.13	19.85±19.48
	Average	42.560±30.330	39.125±25.158	30.405±16.667	35.195±28.366
Females					
PND 22	Block 1	17.80±10.92	21.26±13.72	14.48±4.54	19.50±8.58
	Block 2	13.42±11.64	14.33±11.70	10.47±6.89	14.22±8.46
	Block 3	11.02±7.56	12.85±7.34	11.10±9.58	14.98±12.52
	Block 4	12.05±10.43	12.45±8.58	10.89±9.01	15.94±12.68
	Block 5	10.66±9.03	11.24±7.62	11.28±10.69	18.13±17.91
	Average	12.980±8.916	14.420±8.010	11.645±7.183	16.555±11.031
PND 62	Block 1	38.34±36.65	38.99±25.53	28.76±15.66	37.23±17.77
	Block 2	25.48±25.24	23.72±16.61	16.10±9.01	20.11±16.63
	Block 3	16.30±12.99	13.31±11.94	15.07±12.16	19.52±14.51
	Block 4	18.95±20.81	15.94±12.13	10.26±8.60	17.31±11.37
	Block 5	15.72±12.81	15.44±15.98	14.67±17.21	17.24±14.75
	Average	22.955±19.887	21.485±13.746	16.975±9.750	22.280±13.290

a Data (n=20), obtained from pages 443-444 of MRID 46151802, were presented in grams (g).
b Block=10 consecutive trials
c Response magnitude = Peak response- Baseline response
Average peak response for 10 trials/block for the 20 animals in Subset 3 are presented in raw data tables, F5 (pp. 1-8), Appendix F, pp. 509-524, MRID 46151802.

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d. Learning and memory testing - No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance and water maze tests (Tables 9a and 9b).

Table 9a. Passive avoidance performance (mean±SD) in F_1 rats in Subset 2. [a]

Session/Parameter		Dose (mg/kg/day)			
		0	0.5	1	10
Males					
Session 1 Learning PNDs 22-24	Trials to criterion	4.6±1.8	4.2±0.8	4.9±1.7	4.5±1.3
	Latency trial 1 (sec)	10.0±5.2	7.4±3.8	7.6±5.4	7.6±3.6
	Latency trial 2 (sec)	30.1±21.5	31.9±19.5	27.9±21.9	31.0±20.8
	Failed to learn	0 (0.0)	0 (0:0)	0 (0.0)	0 (0.0)
Session 2 Retention PNDs 29-31	Trials to criterion	2.6±0.7	3.0±0.8	2.8±1.0	3.0±0.8
	Latency trial 1 (sec)	32.2±26.4	26.6±25.9	39.4±25.8	37.5±22.2
Females					
Session 1 Learning PNDs 22-24	Trials to criterion	4.4±1.3	3.9±0.8	4.2±1.1	4.3±0.6
	Latency trial 1 (sec)	8.1±3.2	10.6±5.5	7.8±4.0	10.6±7.0
	Latency trial 2 (sec)	29.3±17.7	41.7±23.1	34.7±23.6	32.8±20.7
	Failed to learn	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Session 2 Retention PNDs 29-31	Trials to criterion	2.8±0.5	2.8±0.7	3.0±0.5	2.8±0.8
	Latency trial 1 (sec)	34.8±22.0	37.3±24.2	28.2±23.3	37.2±24.6

a Data (n=19-20) were obtained from page 416 of MRID 46151802.

Table 9b. Water maze performance (mean±SD) in F_1 rats in Subset 2.[a]

Session/Parameter		Dose (mg/kg/day) 0	0.5	1	10
Males					
Session 1 Learning PNDs 58-62	Trials to criterion	9.4±3.1	9.6±3.0	10.4±2.7	11.0±3.2
	Errors/trial	0.44±0.26	0.36±0.18	0.45±0.23	0.40±0.18
	Latency trial 2 (sec)	15.3±13.1	15.2±9.4	11.2±6.0	15.6±7.9
	Failed to learn	2 (10.0)	0 (0.0)	0 (0.0)	2 (10.5)
Session 2 Retention PNDs 65-69	Trials to criterion	7.1±2.9	6.8±3.0	7.2±2.8	7.0±2.8
	Errors/trial	0.08±0.10	0.15±0.20	0.14±0.16	0.15±0.18
	Latency trial 1 (sec)	7.6±3.0	11.6±7.0	8.9±3.8	11.2±5.2
Females					
Session 1 Learning PNDs 58-62	Trials to criterion	10.4±3.3	9.4±3.2	10.5±3.1	10.0±3.1
	Errors/trial	0.44±0.19	0.46±0.32	0.36±0.14	0.47±0.17
	Latency trial 2 (sec)	14.4±8.6	14.8±7.6	10.8±4.9	15.2±12.4
	Failed to learn	3(15.8)	2(10.0)	3(15.8)	1(5.3)
Session 2 Retention PNDs 65-69	Trials to criterion	6.6±2.0	8.2±2.7	5.9±1.5	6.6±2.5
	Errors/trial	0.16±0.17	0.22±0.14	0.08±0.12	0.09±0.10
	Latency trial 1 (sec)	13.7±5.6	16.4±10.2	9.8±4.5	9.9±6.6

a Data (n=16-20) were obtained from page 417 of MRID 46151802.

5. Postmortem results

a. Brain weights - No treatment-related effect was observed on brain weights of offspring (Tables 10a and 10b).

Table 10a. Mean (±SD) absolute and relative to body brain weights in F_1 rats from Subset 1 at PND 21.[a]

Weight	Dose (mg/kg)			
	0	0.5	1	10
Males				
Fresh Tissue Weight				
Terminal Body (g)	44.0±7.1	48.7±9.1	46.4±9.2	45.4±9.1
Absolute Brain (g)	1.358±0.146	1.382±0.163	1.458±0.191	1.416±0.181
Relative Brain (%)	3.138±0.438	2.942±0.669	3.209±0.413	3.189±0.473
Fixed Tissue Weight				
Terminal Body (g)	49.4±7.6	51.5±5.6	43.9±10.0	49.4±7.2
Absolute Brain (g)	1.647±0.143	1.683±0.103	1.578±0.150	1.688±0.087
Relative Brain (%)	3.371±0.302	3.290±0.248	3.699±0.553	3.466±0.390
Females				
Fresh Tissue Weight				
Terminal Body (g)	46.1±7.1	50.6±4.2	44.6±6.5	48.4±4.7
Absolute Brain (g)	1.377±0.180	1.448±0.149	1.359±0.099	1.398±0.146
Relative Brain (%)	3.039±0.574	2.878±0.365	3.096±0.407	2.898±0.297
Fixed Tissue Weight				
Terminal Body (g)	43.1±7.1	49.0±4.6	44.5±8.1	44.8±6.9
Absolute Brain (g)	1.499±0.132	1.624±0.061	1.570±0.147	1.547±0.098
Relative Brain (%)	3.533±0.422	3.332±0.263	3.610±0.529	3.503±0.388

a Data (n=10) were obtained from pages 405-406 of MRID 46151802.

Table 10b. Mean (±SD) absolute and relative to body brain weights in F_1 rats from Subset 4 at PND 71.[a]

Weight	Dose (mg/kg)			
	0	0.5	1	10
Males				
Terminal Body (g)	416.1±35.8	414.0±39.8	378.8±31.3	426.1±42.5
Absolute Brain (g)	2.209±0.165	2.217±0.148	2.124±0.133	2.289±0.088
Relative Brain (%)	0.531±0.030	0.538±0.031	0.563±0.035	0.540±0.038
Females				
Terminal Body (g)	258.1±29.5	267.0±30.0	245.7±14.1	253.9±20.4
Absolute Brain (g)	2.012±0.108	2.116±0.117	2.069±0.100	2.081±0.138
Relative Brain (%)	0.784±0.061	0.797±0.078	0.841±0.034	0.823±0.066

a Data (n=9-10) were obtained from pages 526-527 of MRID 46151802.

b) Neuropathology

1) Macroscopic examination - No treatment-related gross pathological findings were noted in any treated group at either PND 22 or 70. The length of the cerebellum (anterior to posterior) was decreased ($p \leq 0.05$) in the 1 mg/kg/day males (6.8 mm treated vs 7.3 mm controls). Other gross linear measurements in the treated groups were similar to controls.

2) Microscopic examination - No treatment-related histopathological findings or microscopic linear brain measurements (Table 11) were noted in any treated group at either PND 22 or 70. Hydrocephalus was observed in one 10 mg/kg/day male rat at PNDs 22 (mild severity), and 70 (minimal severity), and two controls at PND 70 (minimal to mild severity). Minimal sciatic nerve fiber degeneration was observed in one control male rat at PND 70. Minimal tibial nerve degeneration (2 control males and 2 control females and 1 high-dose female) and minimal peroneal/sural nerve degeneration (one 10 mg/kg/day female and 2 control females) were observed at PND 70. No other microscopic lesions were noted.

Table 11. Microscopic linear brain measurements[a]

Area length (μ)	Dose (mg/kg/day)	
	0	10
Males		
Day 22		
Frontal cortex	1851±67.9	1809±87.2
Parietal cortex	1797±51.9	1779±69.4
Striatum (caudate putamen)	2649±61.7	2607±83.0
Corpus callosum	170±16.5	174±25.0
Hippocampus	1296±69.0	1341±70.8
Cerebellum	4842±155.0	4866±188.6
Day 70		
Frontal cortex	1902±106.0	1896±74.6
Parietal cortex	1950±56.5	1911±47.0
Striatum (caudate putamen)	3226±123.5	3326±124.0
Corpus callosum	242±45.5	268±31.4
Hippocampus	1500±81.2	1521±63.3
Cerebellum	5346±274.9	5544±208.2
Females		
Day 22		
Frontal cortex	1794±52.5	1794±70.4
Parietal cortex	1776±61.3	1776±77.2
Striatum (caudate putamen)	2574±107.5	2580±82.5
Corpus callosum	169±25.9	181±34.1
Hippocampus	1263±43.5	1269±73.6
Cerebellum	4692±216.9	4632±83.9
Day 70		
Frontal cortex	1854±59.7	1857±51.9
Parietal cortex	1851±84.9	1854±56.2
Striatum (caudate putamen)	3178±131.6	3216±108.5
Corpus callosum	245±11.6	249±34.2
Hippocampus	1461±61.7	1458±56.9
Cerebellum	5262±236.7	5226±270.5

a Data (n=10) were obtained from pages 704-707 of MRID 46151802.

c) <u>Cholinesterase determinations</u> - ChE levels in male and female pups individually and combined at PNDs 4 and 21 are presented in Tables 12 a-c. No significant or ≥20% deceases in ChE activity in the brains, plasma or RBCs of male or female pups were seen at PND 4. There were, however, slight decreases in male and female plasma ChE at 1 and 10 mg/kg/day (males: 9 or 11% ↓, respectively; females: 15 or 14% ↓, respectively); whether these decreases have biological relevance is unclear. Thus, the lack of an unambiguous effect on ChE inhibition in the PND 4 pups is a concern because there may have been an absence of exposure during gestation and/or early lactation.

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In contrast to the investigator's claim that "biologically" important reduction(s) ChE in the brain, plasma and RBCs of the F1 generation rats on PND 21 only occurred in the high-dose males of the 10-mg/kg/day group, our reviewers note significant ($p<0.01$) and dose-related reductions in male brain ChE ranging from a 29 % ↓at 0.5 mg/kg/day, 34% ↓ at 1 mg/kg/day to a 62% ↓ at 10 mg/kg/day on PND 21. There were also significant ($p<0.01$) reductions in plasma (46% ↓) and RBC (50% ↓) ChE in the high-dose males at PND 21. The investigators also claimed that "statistical significance occurred for only one sex". However, our reviewers noted a 25% reduction in brain ChE at 0.5 and 1 mg/kg/day and a significant ($p<0.5$), 58 % decrease at 10 mg/kg/day for PND 21 in the female pups . The lack of a significant effect at the low and intermediate doses was likely due to the marked variability in the vehicle control data as indicated by the high standard deviation. There was also a significant decrease in plasma and RBC ChE in the 10-mg/kg/day group at PND 21.

Table 12a. Mean (± SD) cholinesterase activity in male pups at PNDs 4 and 21.[a]

Cholinesterase activity	Dose (mg/kg/day)			
	0	0.5	1	10
PND 4				
Brain (U/g)	3.0317±0.5990	2.8687±0.5788	2.9466±0.8506	2.8069±0.4018
Plasma (U/mL)	0.7169±0.1123	0.6955±0.1198	0.6554±0.1130	0.6443±0.1171
Erythrocyte (U/mL)	1.9673±0.2147	1.8054±0.3459	1.8153±0.4610	1.8721±1.0498
PND 21				
Brain (U/g)	7.0613±0.8717	5.0326±0.7289 ** (29%↓)	4.6790±1.0565** (34%↓)	2.6681±0.4785 ** (62%↓)
Plasma (U/mL)	0.5266±0.0694	0.5011±0.1087 (5%↓)	0.5160±0.1249 (2%↓)	0.2828±0.0568 ** (46%↓)
Erythrocyte (U/mL)	1.2133±0.4582	0.9373±0.2834 (23%↓)	1.0252±0.2060 (16%↓)	0.6015±0.1672 ** (50%↓)

Table 12b. Mean (± SD) cholinesterase activity in female pups at PNDs 4 and 21.[a]

Cholinesterase activity	Dose (mg/kg/day)			
	0	0.5	1	10
PND 4				
Brain (U/g)	3.0007±0.5274	2.7622±0.4790	3.0366±0.6168	2.9349±0.6492
Plasma (U/mL)	0.7634±0.1112	0.6463±0.1298	0.6505±0.1508	0.6590±0.1063
Erythrocyte (U/mL)	1.5406±0.7024	2.0746±0.6541	1.5386±0.4306	1.4810±0.2724
PND 21				
Brain (U/g)	6.9591±2.6026	5.1930±0.7204 (25%↓)	5.1642±0.9333 (25%↓)	2.9310±1.0674 * (58%↓)
Plasma (U/mL)	0.5604±0.0636	0.4344±0.0988 (23%↓)	0.4721±0.1232 (16%↓)	0.3181±0.0901 ** (43%↓)
Erythrocyte (U/mL)	1.1770±0.4396	1.0096±0.3026 (14%↓)	0.9516±0.3130 (19%↓)	0.4365±0.1786 ** (63↓)

a Data obtained from pages 776-777 of MRID 46151802.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Table 12c. Mean (± SD) cholinesterase activity in combined male and female pups at PNDs 4 and 21.[a]

Cholinesterase activity	Dose (mg/kg/day)			
	0	0.5	1	10
PND 4				
Brain (U/g)	3.0168±0.5604	2.8138±0.5282	2.9832±0.7596	2.8774±0.5520
Plasma (U/mL)	0.7376±0.1111	0.6752±0.1225	0.6527±0.1316	0.6508±0.1094
Erythrocyte (U/mL)	1.7397±0.5612	1.9310±0.5124	1.6616±0.4535	1.6881±0.7900
PND 21				
Brain (U/g)	7.0102±1.8898	5.0326±0.7289 ** (28%↓)	4.9216±1.0016** (30%↓)	2.7926±0.7994 ** (60%↓)
Plasma (U/mL)	0.544±0.0668	0.4678±0.1067 * (14%↓)	0.4952±0.1227 (9%↓)	0.3005±0.0755 ** (45%↓)
Erythrocyte (U/mL)	1.1961±0.4373	0.9754±0.2879 * (18%↓)	0.9949±0.2490 (17%↓)	0.5190±0.1885 ** (57%↓)

a Data obtained from pages 776-777 of MRID 46151802.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that the NOAEL for both F0 and F1 generation rats for all parameters evaluated was 10 mg/kg/day. However, "Biologically important reductions of ChE in the brain, plasma and RBCs of the F1 generation rats as measured on PND 21 occurred at the high dose of 10 mg/kg/day".

B. REVIEWER'S COMMENTS - No treatment-related effect was observed on maternal mortality, clinical signs, abbreviated functional observations, body weight, food consumption, reproductive performance, and gross pathology.

Treatment had no adverse effects on offspring survival, body weight, body weight gain, food consumption, clinical signs, FOB, developmental landmarks, auditory startle reflex, learning and memory, brain weights, brain morphology or neuropathology. Assessment for motor activity revealed a non-significant but dose-related decrease in the number of movements (19% ↓at 1 mg/kg/day to 30%↓at 10 mg/kg/day) that was accompanied by non-significant but comparable dose-related decreases in time spent in movement (19% ↓at 1 mg/kg/day to 28%↓at 10 mg/kg/day) in females on Day 21. However, it was determined that no conclusions can be drawn regarding the effect of acephate on motor activity because the variability in the data was so high.

No treatment-related cholinesterase inhibition (ChEI) was seen in the brains, plasma or red blood cells of male or female pups at PND 4. On Day 21, dose-depended and statistically significant ChEI of the brain were seen. Inhibition at the low, mid and high dose groups were 29 %, 34% and 62%, respectively, in males and 25%, 25%, and 58%, respectively, in females. There were also significant ($p<0.01$) reductions in plasma (46% in males and 43% in female) and RBC (50% in males and 63% in females) ChEI in males at the high-dose males at PND 21.

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The maternal NOAEL is 10 mg/kg/day (HDT). A maternal LOAEL is not established.

The offspring LOAEL is 0.5 mg/kg/day (LDT), based on statistically significant and dose-depended inhibition of brain cholinesterase activity in male and female pups on Day 21. An offspring NOAEL was not established.

This study is classified **Acceptable/NonGuideline** 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 inadequacies in the assessment of motor activity in the offspring and the pending comprehensive review of the positive control data.

C. STUDY DEFICIENCIES -

- Inadequate assessment of motor activity.
- Details concerning the FOB were not provided.
- Formulation analyses data for homogeneity and stability were not provided.