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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC - 3 1997

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

November 19, 1997

MEMORANDUM

SUBJECT: ORTHENE® Technical (Acephate): Review of the Neurotoxicity Studies with Rats: Acute Neurotoxicity Screening Battery (81-8), Two Acute Range-Finding Studies and a Subchronic (13 Weeks) Study (82-7).

Rereg. Case No. 0042 Chemical Code No. 103301
CAS Reg. No. 30560-19-1 Tox. Chem. No. 002A

Sponsor: Valent U.S.A. Corporation, Walnut Creek, CA.

FROM: Krystyna K. Locke, Toxicologist
Toxicology Branch II
Health Effects Division (7509C)

KK Locke 11/19/97

TO: Larry Schnaubelt, PM 72
Special Review and Reregistration Division (7508W)

THRU: Stephen C. Dapson, Branch Senior Scientist
Toxicology Branch II
Health Effects Division (7509C)

Stephen C. Dapson 12/1/97

Toxicology Branch II/HED has completed an evaluation of the following studies:

Guideline No.	MRID No.	DP Barcode	Acceptability
81-8	44203303	D233251 for	Yes
82-7	44203304	all studies	Yes
None	44203301	listed in this	Yes ■
None	44203302	table.	Yes ■

Yes = Acceptable-Guideline.

Yes ■ = Acceptable-Nonguideline. These are acute range-finding studies which have detailed summaries but no separate DERs. The summaries have been included as ATTACHMENT I in the DER for the main acute study (MRID 44203303).

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Chemical:	**Acephate**
PC Code:	**103301**
HED File Code	**13000 Tox Reviews**
Memo Date:	**12/03/1997**
File ID:	**TX012416**
Accession Number:	**412-01-0170**

HED Records Reference Center
05/22/2001

Results obtained in the two guideline studies are summarized below.

MRID 44203303 (Acute Neurotoxicity Screening Battery; Rat) - In this study, ORTHENE® Technical (Acephate; purity: 99%, but assumed to be 100% for dose calculation purposses) was administered by gavage to male and female Sprague-Dawley rats at single doses of 0, 10, 100 or 500 mg/kg. The doses used were based on the results of two range-finding studies (MRID 44203301 and 44203302).

The following treatment-related findings were observed in the 500 and 100 mg/kg male and female groups: **(1)** Whole body and/or limb tremors; ataxia, weakness in hindlimbs and repetitive movement of mouth and jaws; alterations in posture, gait and mobility; low arousal and no approach and touch responses; decreased rearing and activities, rotarod performance, and body temperature; increased righting reflex and time to first step; and salivation, lacrimation and soiled fur; **(2)** Decreased body weight gains in males only; and **(3)** Inhibition of cholinesterase (ChE) activities in plasma, erythrocytes (RBC) and brain (the six regions tested). Findings observed only in the 500 mg/kg male and female groups were: Increased catalepsy time and clonic convulsions; absence of the pinch, startle, pupil and olfactory responses; decreased hindlimb footsplay and forelimb and hindlimb grip strength; chromodacryorrhea; and clear or colored staining/matting material on various body surfaces.

The following treatment-related findings were observed in the 10 mg/kg group: Whole body tremors (single occurences) in one male and one female; inhibition of ChE activities in plasma, RBC and brain regions, in males and females; and decreased rotarod performance in males.

Based on the neurotoxic effects, the LOEL and NOEL for neurotoxicity, for both sexes, are 10 mg/kg (LDT) and <10 mg/kg, respectively. The LOEL and NOEL for the inhibition of ChE activities in plasma, RBC and brain are also 10 mg/kg and <10 mg/kg, respectively. This study is classified as ACCEPTABLE-Guideline and satisfies the guideline requirement for an acute neurotoxicity study in the rat (81-8).

MRID 44203304 (Subchronic Neurotoxicity Study; Rat) - In this study, ORTHENE® Technical (Acephate; purity: 99%, but assumed to be 100% for dose calculation purposes) was administered in the diet to Sprague-Dawley rats for 13 weeks. The nominal doses used (0, 5, 50 or 700 ppm) were based on the results of two earlier dietary studies with ORTHENE® Technical: a 13-week cholinesterase (ChE) inhibition study with rats (MRID 40504819) and a rat feeding/carcinogenicity study (MRID 00084017). The mean intake of ORTHENE® Technical in the current study was 0, 0.33, 3.31 and

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48.63 mg/kg/day, respectively, for males and 0, 0.41, 3.95 and 58.21 mg/kg/day, respectively, for females.

The following findings were observed in the 700 ppm group: (1) Significant inhibition of ChE activities in plasma, erythrocytes (RBC) and brain (the six regions tested) in both sexes; (2) Decreased body weight (males) and body weight gain (males and females); (3) Increased food consumption (when measured as g/kg/day) in both sexes; (4) Increased grooming and rearing, and decreased rotarod time in males; and (5) Decreased motor activity in females.

At the 50 ppm dose, there was a significant inhibition of brain ChE activity (all regions) in both sexes, at all testing intervals (weeks 3, 7 and 13). Plasma ChE activity was significantly inhibited, also in both sexes, only during week 3. Erythrocyte ChE activity was not singificantly inhibited at all testing intervals. Other effects seen in this group included a slight increase in clinical signs, especially hair loss on the forelimbs and at the base of tail.

The only toxic sign observed in the 5 ppm group was the significant inhibition of brain ChE activity.

Based on increases in clinical signs, the LOEL and NOEL for systemic toxicity are 50 ppm (mg/kg/day: 3.31/3.95 ♂/♀) and 5 ppm (mg/kg/day: 0.33/0.41 ♂/♀), respectively.

Based on the findings of Functional Observational Battery (FOB) testing and decreased motor activity, the LOEL and NOEL for neurotoxicity are 700 ppm (mg/kg/day: 48.63/58.27 ♂/♀) and 50 ppm (mg/kg/day: 3.31/3.95 ♂/♀),respectively.

The LOELs for the inhibition of ChE activities in plasma, RBC and brain are 50, 700 and 5 ppm, respectively (mg/kg/day: 3.31/3.95, 48.63/58.27 and 0.33/0.41 ♂/♀, respectively). The NOEL for the inhibition of ChE activities in plasma, RBC and brain are 50, 5 and <5 ppm, respectively (mg/kg/day: 3.31/3.95, 0.33/0.41 and <0.33/0.41 ♂/♀, respectively).

This study is classified as ACCEPTABLE-Guideline and satisfies the guideline requirement for a subchronic neurotoxicity study in the rat (82-7).

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Acephate (Orthene® Technical) Acute Neurotoxicity (81-8)

Primary Review by: Krystyna K. Locke, Toxicologist
Toxicology Branch II, Health Effects Division (HED) KK Locke 8/7/97

Secondary Review by: Kathleen Raffaele, Toxicologist
Toxicology Branch II/HED Kathleen C. Raffaele 8/14/97

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Screening Battery (81-8) and Two Range-Finding Studies

EPA IDENTIFICATION NUMBERS:

MRID No. 44203303 (Main Study) Rereg. Case No. 0042
MRID No. 44203301 and 44203302 (Range-Finding Studies)
DP Barcode No. D233251 Case No. 819371
Submission No. S518374 P.C./I.D. No. 103301
CAS Reg. No. 30560-19-1 Tox. Chem. No. 002A

TEST MATERIAL: ORTHENE® Technical (Acephate; 0,S-Dimethyl acetylphosphoramidothioate), obtained from Valent Dublin Lab, Dublin, CA; white powder, water-soluble; purity: 99%; lot number: SX1725; stable when stored frozen in a desiccator, under nitrogen, protected from light.

SPONSOR: Valent U.S.A. Corporation, Walnut Creek, California

STUDY NUMBER: WIL-194013

TESTING FACILITY: WIL Research Laboratories, Inc., Ashland, Ohio

TITLE OF REPORT: An Acute Neurotoxicity Study of ORTHENE® Technical in Rats

AUTHOR: Mark D. Nemec

STUDY COMPLETION DATE: December 9, 1996

EXECUTIVE SUMMARY: In the acute neurotoxicity study (81-8; MRID 44203303), ORTHENE® Technical (acephate; purity: 99%) was administered in a single gavage dose to groups of 30 male and 30 female non-fasted Sprague-Dawley rats (Crl:CD® BR strain). The doses used (0, 10, 100 or 500 mg/kg) were based on the results of two range-finding studies (MRID 44203301 and 44203302) and were administered as solutions in deionized water. Parameters examined included: **(1)** Daily observations for changes in clinical condition - for all animals; **(2)** Body weights before dosing, on dosing day (day 0), and on days 7 and 14 or 15 after dosing - for all animals; **(3)** Functional observational battery (FOB), for 12 animals/sex/group - before dosing, at 2.5 hours after dosing ("peak effect"), and on study days 7 and 14; **(4)** Locomotor

activity (MA), after the completion of the FOB; **(5)** Cholinesterase (ChE) activities in plasma, erythrocytes (RBC) and 6 brain regions (brain stem, cerebellum, cortex, hippocampus, midbrain and olfactory), for 6 animals/sex/group/sampling time - before dosing, at 2.5 hours after dosing, and on study days 7 and 14; **(6)** Whole and regional brain weights for all ChE animals; **(7)** Whole brain weights and brain dimensions for the FOB/MA animals; and **(8)** Microscopic examination of selected central and peripheral nervous tissues from 5 animals/sex in the control and 500 mg /kg FOB/MA group, at the termination of the study (day 15).

The following treatment-related findings were observed in the 500 mg/kg and 100 mg/kg male and female groups: **(1)** Whole body and/or limb tremors; ataxia, weakness in hindlimbs and repetitive movement of mouth and jaws; alterations in posture, gait and mobility; low arousal and no approach and touch responses; decreased rearing and motor activities, rotarod performance, and body temperature; increased righting reflex and time to first step; and lacrimation, salivation and soiled fur; **(2)** Decreased body weight gains in males only (41-45% and 15% in the high-dose and mid-dose groups, respectively); and **(3)** Inhibition of cholinerase activities in plasma (86-88%), RBC (53-55%) and brain (the six regions tested: 83-88%). Findings observed only in the 500 mg/kg male and female groups were: Increased catalepsy time and clonic convulsions; absence of the pinch, startle, pupil and olfactory responses; decreased hindlimb footsplay and forelimb and hindlimb grip strength; chromodacryorrhea; and clear or colored (tan, red, brown and/or yellow) staining/matting material on various body surfaces.

The following treatment-related findings were observed in the 10 mg/kg male and female groups: Whole body tremors (single occurrences) in one male and one female; inhibition of ChE activities in plasma (31-34%), RBC (18-19%) and brain regions (37-48%); and decreased rotarod performance in males on day 0 (when compared with that of the controls).

Toxic signs occurred within 0.5-2.5 hours after dosing and persisted for 4-8 hours or longer, but were not observed during the next day (study day 1). Plasma and RBC ChE activities were inhibited significantly ($p<0.01$) only during the dosing day. Brain ChE activities were inhibited ($p<0.01$) during dosing day (all regions), day 7 after dosing (all regions but olfactory) and day 14 (midbrain only). Other parameters examined in this study were not affected by ORTHENE® Tech.

Based on the above findings, the LOEL and NOEL for neurotoxicity, for both sexes, are 10 mg/kg (LDT) and <10 mg/kg , respectively. The LOELs and NOELs for the inhibition of plasma, RBC and brain cholinesterase activities are also 10 mg/kg and <10 mg/kg,

respectively. This study is ACCEPTABLE and satisfies the guideline requirement for an acute neurotoxicity study in the rat (81-8).

EXPERIMENTAL PROCEDURES

This study was conducted during November 27, 1995 and February 25, 1996. Non-fasted male and female Sprague-Dawley rats (Crl: CD®BR strain), 30/sex/dose, received single gavage doses of ORTHENE® Technical (Acephate; not corrected for purity) as follows: 0 (vehicle), 10, 100 or 500 mg/kg of body weight. ORTHENE® Technical (purity: 99%) was administered as solutions in deionized water, using 5 mL/kg of body weight. These solutions (referred to in the submitted report as "dosing formulations" or "test article preparations") were analyzed for the concentration of acephate. For homogeneity and stability analyses, samples were collected from formulations prepared prior to initiation of dosing (not administered to animals). The doses used in the current study were based on the results of two range-finding studies (MRID 44203301 and 44203302) in which 14 single doses of ORTHENE® Technical (0.5-900 mg/kg) were tested and which are summarized in ATTACHMENT I of this review. In the current study, the rats were:

(1) Obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan.

(2) Acclimated for at least 18 days before the initiation of dosing.

(3) Young adults, 43-48 days old, and weighing 164-250g (males) and 116-176g (females) at the initiation of dosing.

(4) Assigned to groups using a computer-based randomization procedure which ensured homogeneity of group means and variances for body weight.

(5) Housed individually at 72-75°F, relative humidity of 33-52% and a photoperiod of 12 hours light/12 hours dark.

(6) Identified by cage cards and metal eartags displaying the animal's number.

(7) Fed unrestricted amounts of food (Purina® Certified Rodent Chow #5002) and municipal water.

The following parameters were examined:

(1) Clinical Observations: Daily, throughout the study, for all animals.

(2) Body Weight (for each rat): Before dosing (day -7), on dosing day, and on days 7 and 14 or 15 after dosing, for all animals.

(3) Functional Observational Battery (FOB), for 12 animals/sex /group: Before dosing, at 2.5 hours after dosing ("peak effect"), and on study days 7 and 14. These tests included:

(a) Home Cage Observations: Posture, convulsions/tremors, feces consistency, biting and eyelid closure.

(b) Handling Observations: Ease of removal animal from cage, lacrimation/chromodacryorrhea, piloerection, salivation, fur appearance, respiratory rate/ character, mucous membranes/eye/skin color and muscle tone.

(c) Open Field Observations: Mobility, rearing, tremors/ convulsions, grooming, time to first step (seconds), gait, arousal and urination/defecation.

(d) Sensory Observations: Approach, startle, pupil, touch, tail pinch and eyeblink responses; forelimb and hindlimb extensions; air righting reflex; and olfactory orientation.

(e) Neuromuscular Observations: Hindlimb extensor strength and foot splay; grip strength - hind and forelimb; and rotarod performance.

(f) Physiological Observations: Catalepsy, body weight and body temperature.

(4) Locomotor Activity (MA), for 12 animals/sex/group, after the completion of the FOB. Each animal was tested separately in a computer-controlled system utilizing a series of infrared photobeams. Data were collected in one-minute epochs (print intervals) and the duration of the test session was 41 minutes. Because the first epoch was often incomplete (due to the placement of the animal in the activity cage), the first minute of data was discarded for each animal. The remaining 40 minutes of data collection (divided into 10-minute subsessions) were compiled for data presentation.

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(5) Cholinesterase (ChE) Activities in plasma, erythrocytes (RBC) and brain (6 separate regions), for 6 animals/sex/group/sampling time: Before dosing, at 2.5 hours after dosing, and on study days 7 and 14. Rats used for ChE determination were sacrificed by exsanguination under anesthesia. ChE activities were determined by a modification of the Ellman procedure[a]. The homogenates of the following freshly-dissected brain regions were used: olfactory region, cerebellum, hippocampus, cerebral cortex, brain stem and midbrain (including striatum).

[a] Cholinesterase - A modification of the Ellman reaction utilizing 6,6'-Dithiodinicotinic acid and a primary wavelength of 340 nm. Hackthorne, D.R. et al., J.Am.Ind.Hyg.Assoc. 44:547-551 (1983) and Brownson, C. and Watts, D.C., J.Biochem. 131:369-374 (1973).

(6) Scheduled Necropsy Examinations involved the following:

(a) Brain Weights and Measurements: Whole brain and the six regions were weighed for all animals, at all sampling times, which were used for the determination of ChE activities. Whole brains were weighed and also measured (length and width) for all animals which were used in the FOB-MA tests.

(b) Neuropathology: At the termination of the study (observation day 15), the animals used in the FOB/MA tests were sacrificed by exposure to CO_2 atmosphere and then perfused *in situ*. The central and peripheral nervous system tissues were dissected, examined grossly and preserved. The following tissues were examined histopathologically (after embedding in paraffin or plastic, sectioning and staining with hematoxylin and eosin) from 5 animals/sex in the control and 500 mg/kg groups:

Central Nervous System Tissues (embedded in paraffin): Brain (forebrain, center of cerebrum, midbrain, cerebellum and pons, and medulla oblongata); spinal cord, at cervical and lumbar swellings; gasserian ganglion/trigeminal nerves; lumbar dorsal root ganglion and fibers; lumbar ventral root fibers; cervical dorsal root ganglion and fibers; cervical ventral root fibers; optic nerves and eyes.

Peripheral Nervous System Tissues (embedded in plastic): Sciatic nerves (mid-thigh region and

sciatic notch); and sural, tibial and peroneal nerves.

Preserved for Potential Future Examinations: Tail and forelimbs.

Statistical Analyses used were described as follows:

" All statistical tests for data other than Locomotor Activity were performed by a Digital® MicroVAX® 3400 computer with appropriate programming[7]. Analyses were two-tailed (except as noted) for significance levels of 5% and 1%. Each mean was presented with the standard deviation (SD) and the number of animals (N) used to calculate the mean. Body weights, body weight changes, cholinesterase values, absolute and relative brain weights and brain dimensions were analyzed by a one-way analysis of variance (ANOVA). Continuous Functional Observational Battery (FOB) data were analyzed using a one-way ANOVA. Fisher's Exact Test or Dunnett's test was used to compare the control and treated groups. Histopathological findings of the 500 mg/kg group were compared to the control group data by the one-tailed Kolmogorov-Smirnov test[8].

All statistical tests for the Locomotor Activity data were performed using a personal computer installed with SAS/STAT statistical software[9]. Each mean was presented with the standard deviation and the number of animals used to calculate the mean. Locomotor Activity data were analyzed using a two repeated measures ANOVA. If significant treatment effects were observed at a time point, Dunnett's multiple T-test[9] was conducted to determine significant treatment differences from the control group ($p<0.05$)."

[7] BMDP (1979) Biomedical Computer Programs (Dixon, W.J. and Brown, M.B., eds.) University of California Press, Berkeley, CA, pp. 612, 780, 781.

[8] IBM (1971) Scientific Subroutine Package, IBM System.

[9] SAS (1991) SAS/STAT User's Guide, Version 6, 4th Edition. SAS Institute, Cary, North Carolina, 1028 pages.

RESULTS

Analyses of Dosing Preparations

Concentrations of Acephate in dosing solutions (referred to also in the submitted report as "dosing formulations" or "dosing pre-

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parations") were determined by high performance liquid chromatography (HPLC) using an UV detection at 215 nm. The formulations were homogeneous and were stable for 8 days under refrigeration. The concentration of Acephate in these formulations ranged from 99.0 to 99.8% of the target concentrations (page 1136 in the submitted report; MRID 44203303).

Clinical Observations

Similar clinical signs were observed in rats used for the FOB-MA tests and in those used for the determinations of ChE activities. There were no mortalities in this study.

Treatment-related clinical signs were observed in the 500 mg/kg group, and, to a lesser extent, in the 100 mg/kg group. The predominant signs in males and females were: whole body tremors, alterations in posture/gait (body flattened with limbs extended and rocking, lurching or swaying), repetitive movement of mouth, tremors of forelimbs and/or hindlimbs, and twitching of ears. These signs were observed within 0.5-2.5 hours after dosing and persisted for 8 hours or longer after dosing in the 500 mg/kg group and for 4-6 hours after dosing in the 100 mg/kg group. None of these signs were observed during the next day (study day 1).

The following findings were observed at various times following dosing during study day 0 (dosing day) only in the 500 mg/kg group, in both sexes: Salivation, lacrimation and chromodacryorrhea.

Clear or colored (tan, red, brown and/or yellow) staining/matting material on various body surfaces was observed frequently in the 500 mg/kg group, in both sexes, during study days 0-3. Yellow staining/matting material on various body surfaces was also observed less frequently in the 100 mg/kg group on study day 0.

The treatment-related predominant clinical findings, observed in the 500 mg/kg and 100 mg/kg groups, are summarized in **Table I.** The only finding observed in the 10 mg/kg group was a single occurrence of whole body tremors in one male and one female. ■ None of the above findings were observed in the control group.

Table I. Clinical Findings: Number of Rats Affected

ORTHENE® Tech. (mg/kg)	100	500	100	500
Study Group	FOB-MA		Cholinesterase	
No. assigned (♂/♀)	12/12	12/12	18/18	18/18
	30 Minutes After Dosing (♂/♀)			
Tremors, forelimbs	0/0	1/0	0/0	0/0
Tremors, hindlimbs	0/0	2/0	0/0	0/0
Tremors, whole body	0/0	0/2	0/0	0/3
Lacrimation, both eyes	0/0	5/5	0/0	1/0
	1 Hour After Dosing (♂/♀)			
Alterations in gait) ●	0/0	4/4	0/0	5/7
Alterations in posture ●●	0/0	6/3	0/0	8/9
Tremors, whole body	1/0	12/11	2/3	18/17
Tremors, hindlimbs	0/2	0/0	1/2	0/0
Repetitive movement of mouth	0/0	0/0	1/1	3/2
Lacrimation, right eye	0/0	1/3	0/0	0/0
Lacrimation, left eye	0/1	0/3	0/0	0/0
Salivation	0/0	2/9	0/0	2/4
	2 Hours After Dosing (♂/♀)			
Alterations in gait	2/4	1/3	3/2	2/0
Alterations in posture	2/0	9/10	1/2	12/8
Tremors, whole body	12/12	12/11	16/18	17/18
Tremors, forelimbs	0/0	0/1	0/0	0/0
Repetitive movement of mouth	0/0	0/1	0/0	2/2
Lacrimation, right eye	0/0	1/4	0/0	2/7
Lacrimation, left eye	0/0	1/6	0/0	2/7
Salivation	0/0	6/7	0/0	11/11
	4 Hours After Dosing (♂/♀)			
Alterations in gait	0/1	1/1	0/1	2/0
Alterations in posture	1/0	3/4	0/0	4/3
Tremors, whole body	6/4	10/10	10/10	11/12
Repetitive movement of mouth	1/1	2/0	2/2	0/0
Chromodacryorrhea, right eye	0/0	0/1	0/0	0/2
left eye	0/0	2/1	0/0	0/2
Salivation	1/1	2/4	0/0	6/6

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Table I. Clinical Findings: Number of Rats Affected - continued

ORTHENE® Tech. (mg/kg)	100	500	100	500
Study Group	FOB-MA		Cholinesterase	
Lacrimation, right eye	0/0	0/1	0/0	0/4
left eye	0/0	0/1	0/0	0/3
	6 Hours After Dosing (♂/♀)			
Alterations in gait	0/0	4/5	0/0	0/1
Alterations in posture	0/0	3/3	0/0	0/0
Tremors, whole body	0/0	10/7	2/1	10/11
Twitching of ears	0/0	0/0	0/3	0/0
Chromodacryorrhea,				
right eye	0/0	8/3	0/0	0/1
left eye	0/0	7/3	0/0	0/2
Salivation	0/0	1/0	0/0	1/2
Lacrimation, right eye	0/0	0/1	0/0	3/0
left eye	0/0	0/1	0/0	2/0
	8 Hours After Dosing (♂/♀)			
Alterations in gait	0/0	4/3	0/0	1/0
Alterations in posture	0/0	2/1	0/0	0/2
Tremors, whole body	0/0	6/4	0/0	9/9
Tremors, forelimbs	0/0	1/1	0/0	0/1
Tremors, hindlimbs	0/0	1/1	0/0	0/1
Repetitive movement of mouth	0/0	0/1	0/0	0/0
Twitching of ears	0/0	0/0	0/0	0/1
Chromodacryorrhea,				
right eye	0/0	3/4	0/0	1/1
left eye	0/0	3/3	0/0	1/1
Lacrimation, right eye	0/0	0/0	0/0	0/1

This table is based on TABLES 3-6 (pp. 65-74), TABLES 9-10 (pp. 81-86) and TABLES 13-18 (pp. 93-117) in the submitted report (MRID 44203303).

■ Whole body tremors were observed at 1.5 hours after dosing in the female rat and at 2.5 hours after dosing in the male rat. No tremors were reported at 2 and 4 hours after dosing in the female and male, respectively.

● Animal rocks, lurches or sways as it walks. ●● Body flattened with limbs extended.

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Body Weights

Statistically significant decreases in group mean body weight gains were observed in the 500 mg/kg (high-dose) and the 100 mg/kg (mid-dose) FOB-MA males during days 0 (dosing day) to 7. Relative to the control value, these decreases were 41% ($p<0.01$) and 15% ($p<0.05$) for the high-dose and mid-dose males, respectively. In the ChE group, statistically significant decreases in body weight gain were observed only in the 500 mg/kg males and also only during the first week after dosing. Relative to the control value, the group mean decrease was 45% ($p<0.01$). The group mean body weight gains of the high-dose and mid-dose females, and the 10 mg/kg (low-dose) males and females, were not affected by ORTHENE® Tech. in this study. The body weight data are summarized in **Table II.**

Table II. Body Weight Gains

ORTHENE® Tech. (mg/kg)		0	10	100	500
Study Days	No. of Rats (♂/♀)	Weight Gain (g) for FOB-MA Group (♂/♀)			
-7-0	12/12	66/26	62/28	62/24	64/27
0-7	12/12	54/18	52/25	46*/21	32**/17
7-14	12/12	49/18	49/18	47/15	56/21
		Weight Gain (g) for ChE Group (♂/♀)			
-7-0	18/18	59/26	63/27	62/26	58/28
0-7	12/12	51/22	51/18	46/20	28**/19
7-14	6/6	51/21	55/23	56/28	65*/27

This table is based on TABLES 21-22, pages 122-125, in the submitted report (MRID 44203303). Day 0 = Dosing day.
* Significantly different from control group at 0.05 level and ** 0.01 level using Dunnett's test.

Functional Observational Battery (FOB)

The only toxic signs observed in the 10 mg/kg group were slight whole body tremors in one male during the study day 0 (dosing day). Whole body tremors were also noted in the same male during the clinical cage observations.

The following findings were observed in the 100 mg/kg and 500 mg/kg male and female groups during the study day 0 (dosing day)

only: **(1)** Whole body tremors and alterations in posture -- during the home cage observations; **(2)** Salivation, lacrimation, changes in muscle tone and fur appearance -- during the handling observations; **(3)** Whole body tremors, clonic tremors of limbs, clonic convulsions, gait alterations, impaired mobility, repetitive movement of mouth and jaw, altered arousal, decreased rearing activity and increased time to first step -- during the open field observations; **(4)** Alterations in tail pinch response and air righting reflex; and alterations in approach, touch, startle, pupil and olfactory responses (in 500 mg/kg group only) -- during the sensory observations; **(5)** Impairments in hindlimb extensor strength and rotarod performance; and reduced hindlimb footsplay, and forelimb and hindlimb grip strength (in 500 mg/kg group only) -- during the neuromuscular observations; and **(6)** Increased mean catalepsy times and decreased body temperatures -- during the physiological observations. The incidences of selected findings are summarized in **Tables III** and **IV.**

Table III. Functional Observational Battery (FOB): Percent Incidence During Day 0 (Dosing Day)

ORTHENE® Tech. (mg/kg)	100	500	100	500
	Males		Females	
No. assigned to group	12	12	12	12
No. tested	12	12	12	12
Home Cage Observations				
Altered posture **(a)**	50*	83*	25	75*
Whole body tremors	92*	100*	100*	100*
Tremors: Impairment of locomotion: Slight	42*	50*	25	25
Moderate/Marked	17	33	0	58*
Severe	0	8	0	8
Handling Observations				
Lacrimation: Slight	17	67*	17	58*
Severe	0	0	0	25
Salivation: Slight	0	17	0	8
Severe	0	25	0	58*
Fur appearance: Soiled	8	50*	17	75*
Open Field Observations				
Impaired mobility:				
Slightly	83*	0	83*	0
Moderately	8	83*	8	67*
Severe	0	17	0	33
Gait alterations: **(b)**	0	67*	0	50*
Ataxia **(c)**	25	33	50*	50*

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Table III. Functional Observational Battery (FOB): Percent Incidence During Day 0 (Dosing Day) - continued

ORTHENE® Tech. (mg/kg)	100	500	100	500
	Males		Females	
Convulsions - Clonic:				
Mouth and jaws **(d)**	8	58*	25	17
Whole body tremors	58*	92*	50*	100*
Tremors: Impairment of locomotion: Slight	50*	17	42*	17
Moderate/Marked	0	75*	8	50*
Severe	0	8	0	33
Altered arousal: Low	42*	75*	17	67*
Very low	8	25	0	25
Sensory Observations				
No approach response	8	50*	0	50*
No touch response	8	58*	0	58*
No startle response	0	50*	0	33
No tail pinch response	0	58*	0	33
No pupil response	0	33	0	75*
No olfactory orientation	0	33	0	17
Altered air righting reflex **(e)**	50*	100*	50*	100*
Neuromuscular Observations				
Weakness in hindlimbs	50*	58*	33	83*

The above table is based on the following TABLES in the submitted report (MRID 44203303): 25-26 (pp. 130-133); 33-34 (pp. 157-168); 41-42 (pp. 204-215); 49-50 (pp.248-255); and 57-58 (pp.284-287).

* Significantly different from the controls at the 0.05 level using Fisher's Exact Test.

(a) Flattened, limbs may be extended.

(b) Hindlimbs splayed or dragging, unable to support weight.

(c) Ataxia, excessive sway, rocks or lurches as proceeds forward.

(d) Repetitive movement of mouth and jaws.

(e) Slightly uncoordinated, lands on side or back.

All of the statistically significant findings were above the historical incidence reported for the Charles River COBS CD rats (MRID 44203303; APPENDIX H; pp. 1428-1437 and 1456-1464).

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Table IV. Functional Observational Battery (FOB): Findings During Day 0 (Dosing Day) Not Listed in Table III

ORTHENE® Tech. (mg/kg)	0	10	100	500
		Males		
Open Field Observations				
Time to first step (sec.)	0.4	0.6	1.3	10.8**
Rearing ■	5.9	5.8	0.8**	0.0**
Neuromuscular Observations				
Grip strength (g):				
Forelimb	623	564	628	363**
Hindlimb	346	347	374	271*
Rotarod perform. (sec.) ●	100	62.7**	20.4**	2.7**
Hindlimb footsplay (mm)	72.1	78.3	62.2	50.2**
Physiological Observations				
Catalepsy (sec.)	0.5	0.7	0.9	9.1*
Body temperature (°C) ◆	38.1	38.0	34.5**	32.6**
		Females		
Open Field Observations				
Time to first step (sec.)	0.8	0.4	1.5	8.8**
Rearing ■	11.8	8.8	1.3**	0.0**
Urination ■■	0.2	0.1	0.3	0.5
Neuromuscular Observations				
Grip strength (g):				
Forelimb	421	507	592**	296*
Hindlimb	303	312	353	245
Rotarod performance (sec.)	69.0	67.2	38.3	5.7**
Hindlimb footsplay (mm)	60.6	63.8	57.3	53.0
Physiological Observations				
Catalepsy (sec.)	0.3	0.3	0.6	6.2**
Body temperature (°C)	38.6	38.8	35.1**	32.7**

This table is based on the following TABLES in the submitted report (MRID 44203303): 41 (pp. 204-208); 57 (pp. 284-285); and 64 (pp. 303-304).

* Significantly different from the controls at the 0.05 level and ** 0.01 level using Dunnett's Test. All of the statistically significant findings were above the historical control incidence (APPENDIX H; pp. 1433-1437 and 1459-1463).

■ Rearing activity was reported in TABLE 41 of the submission (MRID 44203303) only as "Rearing". However, in Figure 3 of the submission (p. 239), rearing activity was reported as "mean response" on a scale of 0-15. Relative to the control value (13.3), a significant decrease in rearing (6.1*) was still observed in the 500 mg/kg females on day 7.

■ Relative to the control value (0.0), a significant increase in urination (6.1*) was observed in the 500 mg/kg females on day 7.

● In the 10 mg/kg male group, mean rotary performance was 37% ($p<0.01$) lower than the control group mean on day 0. Since the pretest mean rotary performance for this group was 28% (statistically insignificant) lower than the control value, the relationship of the 37% decrease to treatment is not clear.

◆ Relative to the control value (38.4°C), a significant decrease in body temperature (37.8°C *) was still observed for the 500 mg/kg males on day 7.

Locomotor Activity

Relative to the control values, the overall mean total motor activity and ambulatory activity were significantly ($p<0.05$) reduced on day 0 for the 100 mg/kg and the 500 mg/kg male and female groups. These data are summarized in **Table V.**

Table V. Intergroup Comparison of Motor Activity on Day 0 (Dosing Day)

ORTHENE® Tech. (mg/kg)	0	10	100	500
Total Activity (counts): ●				
Males	1246	1261	433*	564*
Females	1447	1716	397*	448*
Total Ambulatory Activity (counts): ●●				
Males	754	753	223*	287*
Females	843	1025	227*	204*
Percent Decrease from Controls				
Total Activity:				
Males	-	0	65*	55*
Females	-	0	73*	69*
Ambulatory Activity:				
Males	-	0	70*	62*
Females	-	0	73*	76*

The above table is based on TABLE 68, pages 315-318, in the submitted report (MRID 44203303). * Significantly different from the controls at the 0.05 level using Dunnett's Test. ● Total motor activity was defined as a combination of motor skills (i.e. grooming; interruption of one or two adjacent photobeams) and ●● ambulatory motor activity (iterruption of three or more consecutive photobeams).

Mean total motor and ambulatory activity counts were also lower than control group means on study day 7 for the 500 mg/kg males (22-28%) and females (32%), and on study day 14 for the 500 mg/kg females (25-26%). However, these decreases were not statistically significant.

ORTHENE® Tech. had no effect on the mean total ambulatory and total motor activity counts in the 10 mg/kg male and female groups.

Cholinesterase Activities

Relative to the control values, plasma, red blood cell (RBC) and brain (the 6 regions studied) cholinesterase (ChE) activities were significantly inhibited in all treated male groups on day 0 (dosing day). The statistically significant inhibition of RBC ChE was still observed in the 500 mg/kg male group on day 14, and of brain ChE, on days 7 and 14. Significant inhibitions of ChE activities were also observed in the femals on day 0: plasma, in the 100 mg/kg and 500 mg/kg groups; and RBC and brain, in all treated groups. Brain ChE was also significantly inhibited in the 100 mg/kg and 500 mg/kg females on days 7 and 14. The ChE inhibition data are summarized in **Tables VI** and **VII.**

Table VI. Cholinesterase Activities in Plasma and RBC

ORTHENE® Tech. (mg/kg)		0	10	100	500
Plasma ChE (U/L)					
Day 0:	Males	815	566**	202**	110**
	Females	1587	1042	460**	192**
Day 7:	Males	748	683	745	672
	Females	2091	2275	1775	1421
Day 14:	Males	782	681	737	790
	Females	2406	2174	2117	1437
RBC ChE (U/L)					
Day 0:	Males	3879	3138**	1916**	1753**
	Females	3912	3203**	2071**	1830**

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Table VI. Cholinesterase Activities in Plasma and RBC -continued

ORTHENE® Tech. (mg/kg)		0	10	100	500
Day 7:	Males	3723	3509	3557	3277
	Females	3820	3458	3488	3646
Day 14:	Males	3914	3590	3596	3473*
	Females	3525	3477	3265	3495
Plasma ChE (% Decrease)					
Day 0:	Males	-	31**	75**	86**
	Females	-	34	71**	88**
Day 7:	Males	-	9	0.4	10
	Females	-	0	15	32
Day 14:	Males	-	13	6	0
	Females	-	10	12	40
RBC ChE (% Decrease)					
Day 0:	Males	-	19**	51**	55**
	Females	-	18**	47**	53**
Day 7:	Males	-	6	4	12
	Females	-	9	9	5
Day 14:	Males	-	8	8	11*
	Females	-	1	7	1

This table is based on TABLE 72, pages 337-338, in the submitted report (MRID 44203303). U/L = International Units/ Liter.
* Significantly different from control group at 0.05 level and ** 0.01 level using Dunnett's Test.
Number of rats used per determination: 6 males and 6 females.

Table VII. Cholinesterase Activity in Brain Regions

ORTHENE® Tech. (mg/kg)		0	10	100	500
Brain Region		ChE Activity (U/G) - Day 0			
Hippocampus:	Males	9.20	5.21**	2.07**	1.41**
	Females	8.72	5.31**	2.67**	1.36**
Olfactory:	Males	18.03	9.39**	3.10**	2.22**
	Females	16.97	9.57**	3.36**	2.22**

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Table VII. Cholinesterase Activity in Brain Regions - continued

ORTHENE® Tech. (mg/kg)		0	10	100	500
Brain Region		ChE Activity (U/G) - Day 0			
Midbrain:	Males	17.57	9.98**	3.64**	2.33**
	Females	17.34	10.15**	3.54**	2.53**
Brain Stem:	Males	15.95	9.57**	3.34**	2.04**
	Females	16.69	9.69**	3.64**	2.20**
Cerebellum:	Males	6.99	4.41**	1.94**	1.17**
	Females	7.25	4.44**	1.98**	1.20**
Cortex:	Males	14.62	8.56**	3.19**	2.21**
	Females	14.66	8.67**	3.39**	2.36**
		ChE Activity (U/G) - Day 7			
Hippocampus:	Males	8.77	7.25	8.14	6.98
	Females	8.78	8.30	7.78	7.02*
Olfactory:	Males	16.94	16.66	12.88	13.93
	Females	16.82	17.62	15.96	14.74
Midbrain:	Males	15.98	14.95	14.55	12.40**
	Females	15.94	16.22	14.35	13.28**
Brain Stem:	Males	14.25	13.76	13.64	12.62*
	Females	14.98	14.05	14.26	12.97**
Cerebellum:	Males	6.37	6.28	6.09	5.88*
	Females	6.61	6.18	6.20	5.67**
Cortex:	Males	15.52	14.59	13.94	11.79**
	Females	15.42	14.46*	13.27**	11.66**
		ChE Activity (U/G) - Day 14			
Hippocampus:	Males	9.53	9.85	9.37	8.56
	Females	8.57	9.50	9.03	8.38
Olfactory:	Males	22.13	19.69	17.82	18.27
	Females	17.15	17.34	17.45	15.77
Midbrain:	Males	16.45	15.46*	15.60	14.10**
	Females	15.73	16.65	15.86	14.90

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Table VII. Cholinesterase Activity in Brain Regions - continued

ORTHENE® Tech. (mg/kg)		0	10	100	500
Brain Region		ChE Activity (U/G) - Day 14			
Brain Stem:	Males	14.28	14.31	14.34	14.04
	Females	13.74	14.86	14.34	13.91
Cerebellum:	Males	6.38	6.38	6.10	6.41
	Females	6.14	6.74	6.41	6.13
Cortex:	Males	15.43	15.25	14.48	13.96
	Females	15.00	15.95	14.85	13.45
		ChE Activity (% Decrease) - Day 0			
Hippocampus:	Males	-	43**	77**	85**
	Females	-	39**	69**	84**
Olfactory:	Males	-	48**	83**	88**
	Females	-	44**	80**	87**
Midbrain:	Males	-	43**	79**	87**
	Females	-	41**	80**	85**
Brain Stem:	Males	-	40**	79**	87**
	Females	-	42**	78**	87**
Cerebellum:	Males	-	37**	72**	83**
	Females	-	39**	73**	83**
Cortex:	Males	-	41**	78**	85**
	Females	-	41**	77**	84**
		ChE Activity (% Decrease) - Day 7			
Hippocampus:	Males	-	17	7	20
	Females	-	5	11	20*
Olfactory:	Males	-	2	24	18
	Females	-	0	5	12
Midbrain:	Males	-	6	9	22**
	Females	-	0	10	17**
Brain Stem:	Males	-	3	4	11*
	Females	-	6	5	13**

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Table VII. Cholinesterase Activity in Brain Regions - continued

ORTHENE® Tech. (mg/kg)		0	10	100	500
Brain Region		ChE Activity (% Decrease) - Day 7			
Cerebellum:	Males	-	1	4	8*
	Females	-	6	6	14**
Cortex:	Males	-	6	10	24**
	Females	-	6*	14**	24**
		ChE Activity (% Decrease) - Day 14			
Hippocampus:	Males	-	0	2	10
	Females	-	0	0	2
Olfactory:	Males	-	11	19	17
	Females	-	0	0	8
Midbrain:	Males	-	6*	5	14**
	Females	-	0	0	5
Brain Stem:	Males	-	0	0	2
	Females	-	0	0	0
Cerebellum:	Males	-	0	4	0
	Females	-	0	0	0
Cortex:	Males	-	1	6	9
	Females	-	0	1	10

This table is based on TABLE 74, pages 343-348, in the submitted report (MRID 44203303). U/G = International Units/Gram
* Significantly different from control group at 0.05 level and ** 0.01 level using Dunnett's Test.
Number of rats used per group: 6 males and 6 females.

Brain Weights and Measurements

ORTHENE® Tech. had no effect at any dose level on absolute brain weights and brain region weights, brain and brain region weights relative to final body weights, brain region weights relative to whole brain weights, and brain dimensions (length and width).

Microscopic Examination

Treatment-related microscopic findings were not observed in any of the central or peripheral nervous system tissues examined at the termination of the study (day 15) from 5 male and 5 female rats in the 500 mg/kg group. The only findings observed in this study were **(1)** rosette formation in the retinal neuroepithelium of one control male and female; and **(2)** digestion chambers (degeneration) in the sciatic nerve of one control and one 500 mg/kg males (TABLE 88, pages 399-404, in the submitted report).

COMMENTS

This study is reported clearly and in a great detail (1516 pages, 4 volumes). It was designed and conducted in compliance with **(1)** EPA Test Guidelines for a Neurotoxicity Screening Battery (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10; Neurotoxicity; Series 81-8-SS; March, 1991); and **(2)** GLP Regulations, 40 CFR Part 160 (October 16, 1989). All test procedures used were either referenced or described. Historical control data from WIL Research Laboratories (testing facility) were provided for the following parameters: functional observational battery, motor activity and neuropathology. The following signed and dated statements were also included in the submitted report: No Data Cofidentiality Claims, Compliance, Flagging, Certificate of Authenticity and Quality Assurance.

According to the author of this report (MRID 44203303), the NOEL for neurotoxicity is < 10 mg/kg (LDT). The NOELs for cholinesterase activities in plasma, RBC and brain are also < 10 mg/kg (LDT). Toxicology Branch II/HED agrees with this conclusion.

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ATTACHMENT I

Summaries of Range-Finding Studies: MRID 44203301 and 44203302

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A Range-Finding Acute Study of ORTHENE® Technical in Rats. Mark D. Nemec. WIL Research Laboratories, Inc., Ashland, Ohio. Study No. WIL-194012; Completion Date: October 5, 1994. **MRID 44203301**

Young adult, non-fasted Sprague-Dawley rats (Crl:CD®BR strain) received single gavage doses of ORTHENE® Tech. (acephate; purity: 99.4%; lot number: SX1725) as follows: **PART A:** 0 (deionized water; vehicle), 25, 50, 75, 150, 300, 450, 600 or 900 mg/kg (2♂ and 2♀/dose, for doses 0-450 mg/kg and 1♂ and 1♀/dose for the remaining doses; dosing date: 3/25-26/93); **PART B:** 0, 10 or 500 mg/kg (1♂ and 1♀/ group; dosing date: 4/5/93); and **PART C:** 0, 5 or 500 mg/kg (5♂ and 5♀/group; dosing date; 4/23/93). The rats were observed for 7 days and were sacrificed on day 8. Parameters examined included daily observations for toxic signs, daily detailed clinical examination, body temperatures and weights, and necropsy (performed only on animals found dead or killed moribund).

Both animals in the 900 mg/kg group, one (female) in the 600 mg/kg group and one (male) in the 500 mg/kg group died within 1-3 days after dosing. Toxic signs observed in the nonsurvivers within 15 min. to 6 hours after dosing were: **(1)** Gait alterations (rocking, lurching or swaying, prostration and/or high carriage), tremors (whole body and/or forelimb/hindlimb), salivation, lacrimation, constricted pupils and impaired air righting reflex; **(2)** Reduced forelimb/hindlimb grasp, hypoactivity, hypothermia, swelling of the face and exophthalmus; **(3)** Labored respiration and head twitch; **(4)** Staining (clear, yellow and/or tan) on the forelimbs, urogenital area and around the mouth; and **(5)** Red ocular discharge, red material around the eyes and nose, and decreased urination and defecation. Most of these toxic signs persisted for 8 hours, some (like gait alterations) for 24 hours and labored breathing, until death. Macroscopic examination of the females revealed dark red contents in the ileum and a reddened cortico-medullary junction in each kidney (one female). The 900 mg/kg male had a distended and gas-filled duodenum and jejunum, a hemorrhagic thymus gland, and a reddened and enlarged mediastinal lymph node. No gross lesions were observed in the 500 mg/kg male which apparently died from blood loss caused by a pulled out claw.

Toxic signs observed in the surviving male and female rats were similar to those observed in the nonsurvivers. These signs occurred at dose levels of 25-900 (HDT) mg/kg. No toxic signs were noted at the two other levels of ORTHENE® Tech. tested, 5 and 10 mg/kg. The minimum effect dose levels (LOELs) and the estimated times of peak effect for each predominant sign are summarized in **TABLE A.**

TABLE A. Toxic Signs Observed in Rats Sacrificed on Day 8 After Single Dosing

Toxic Sign	Min. Effect Dose (mg/kg)		Peak Effect (min.)	
	Males	Females	Males	Females
Gait alterations	25	25	90-120	90-120
Tremors	75	50	90	90
Constricted pupils	25	25	3 hrs.	4 hrs.
Lacrimation	50	25	90	90-150
Exophthalmus	50	25	90-150	90-150
Salivation	300	50	2-3 hrs.	2-3 hrs.
Hypoactivity	150	300	6 hrs.	5 hrs.
Impaired air righting reflex	150	150	90	4 hrs.
Decreased body temperature	25	25	2-4 hrs.	2-4 hrs.
Decreased body weight gain	300	150	-	-

This table is based on data reported on pages 26-38 in the submitted report (MRID 44203301).

Based on the above data, the LOEL and NOEL for neurotoxic effects, for both sexes, are 25 mg/kg and 10 mg/kg, respectively, and the highest nonlethal dose is 500 mg/kg. It was, therefore, recommended that **(1)** the highest dose for the main acute neurotoxicity study with rats (81-8; MRID 44203303) should not exceed 500 mg/kg of ORTHENE® Tech. and **(2)** the time of peak effect should be 150 minutes after dosing. These recommendations appear to be supported by data reported in this range-finding study.

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A Range-Finding Acute Study of ORTHENE® Technical in Rats. Mark D. Nemec. WIL Research Laboratories, Inc., Ashland, Ohio. Study No. Wil-194015; Study Date: December 20, 1996. **MRID 44203302**

Young adult, fasted (about 18 hours) Sprague-Dawley rats (Crl: CD®BR strain) received single gavage doses of ORTHENE® Tech. (acephate; purity: 99.0%; lot number: SX1725) as follows: **Phase I:** 0 (deionized water; vehicle), 5, 25, 125 or 500 mg/kg (2♂ and 2♀/group; dosing date: 9/22/95) and **Phase II:** 0, 0.5, 2.5 or 5.0 mg/kg (5♀/group; dosing date: 10/25/95). All rats were killed at 2.5 hours after dosing. Parameters examined included observation for toxic signs, body weights (on day -1, prior to dosing and prior to sacrifice), brain and brain regions weights, and cholinesterase activities (at the termination of the study) in plasma, erythrocytes (RBC), and brain regions (hippocampus, midbrain, brain stem, cerebellum and cortex).

There were no unscheduled deaths in this study; body, brain and brain region weights were not affected at all dose levels; and no clinical signs were observed in the 0.5-5.0 mg/kg groups.

Treatment-related toxic signs in the 25 mg/kg group were tremors of the mouth (repetitive movement) and twitching of both ears. These signs were observed at the terminal sacrifice (2.5 hours after dosing) in one male rat.

The most prominent findings in the 125 mg/kg male and female groups were tremors of the mouth, forelimbs/hindlimbs and/or whole body; altered gait (rocking, lurching or swaying); and salivation and twitching of both ears. These signs were first observed at 1-2 hours after dosing and were still present at study termination.

The most prominent findings in the 500 mg/kg males and females were the same as those observed in the 125 mg/kg group, plus hypothermia and hypoactivity. These signs were also first observed at 1-2 hours after dosing.

Cholinesterase (ChE) activities, determined at the termination of the study (2.5 hours after dosing), were inhibited in a dose-related manner, in males and females, as follows: **(1)** In plasma, at dose levels of 2.5 mg/kg (F) and 5.0 mg/kg (M), and above; **(2)** in RBC, at dose level of 5.0 mg/kg and above; and **(3)** in brain, at dose level of 0.5 mg/kg and above. The ChE inhibition data are summarized in **TABLES AA and AB.**

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TABLE AA. Inhibition of Cholinesterase Activities in Plasma, Erythrocytes and Brain Regions at 2.5 Hours After Dosing (Study Termination) - Phase I

ORTHENE® Tech. (mg/kg)	5		25		125		500	
	Inhibition (%) Relative to Control Values							
Analyses	M	F	M	F	M	F	M	F
Plasma	30	26	55	44	75	70	84	91
Erythrocytes (RBC)	15	13	29	31	45	46	49	50
Brain Regions:								
Hippocampus	32	31	56	63	74	76	78	84
Midbrain	25	30	53	58	71	74	75	80
Brain Stem	28	25	56	60	73	73	78	80
Cerebellum	18	26	50	54	66	71	75	79
Cortex	28	30	55	62	73	75	78	80

This table is based on data reported on page 24 in the submitted report (MRID 44203302). Number of rats used per dose: 2 males (M) and 2 females (F).

Cholinesterase activities (U/L in the case of plasma and RBC, and U/G in the case of brain) were determined by the original Ellman method (1961) and the modified Ellman method (1973, 1983), as follows:

(1) Ellman, G.L.Courtney, K.D., Andres, V. and Fetherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm. 7:88-95.

(2) Borwnson, C. and Watts, D.C. (1973). The modification of cholinesterase activity by 5,5'-dithiobis-(2-nitrobenzoic acid) included in the coupled spectrophotometric assay. Biochem. J. 131:369-374 (referenced as J. Biochem. in the acute neurotoxicity study, MRID 44203303).

(3) Hackathorn, D.R., Brinkman, W.J., Hathaway, T.R., Talbott, T.D. and Thompson, L.R. (1983). Validation of a whole blood method for cholinesterase monitoring. Am. Ind. Hyg. Assoc. J. 44(7):547-551.

Both methods were used because it was originally believed that the modified method was more sensitive than the original method. However, very similar results were obtained by both methods. **TABLE AA** above contains results obtained by the modified method.

TABLE AB. Inhibition of Cholinesterase Activities in Plasma, Erythrocytes and Brain Regions at 2.5 Hours After Dosing (Study Termination) - Phase II

ORTHENE® Tech. (mg/kg)	0.5	2.5	5.0
	Inhibition (%) Relative to Control Values		
Analyses	F	F	F
Plasma	0	14	10
Erythrocytes (RBC)	8	3	19
Brain Regions:			
Hippocampus	8	13	30
Midbrain	4	21	30
Brain Stem	7	22	34
Cerebellum	0	20	33
Cortex	0	21	31

This table is based on data reported on page 26 in the submitted report (MRID 44203302). Number of rats used per dose: 5 females (F). Zero (0) inhibition = ChE activities were the same or greater than those for the respective control groups.

Cholinesterase activities in plasma, RBC and brain regions were determined by the modified method of Ellman (referenced under **TABLE AA** above).

Based on the clinical signs, **the NOEL and LOEL for systemic toxicity**, for both sexes, were 5 mg/kg and 25 mg/kg, respectively. Based on the ChE activities data, the NOELs and LOELs for ChE inhibitions were:

Plasma ChE NOEL = 0.5 mg/kg (F) and < 5.0 mg/kg (M; LDT); **LOEL** = 2.5 mg/kg (F) and 5.0 mg/kg (M).

RBC ChE NOEL = 2.5 mg/kg (F) and < 5.0 mg/kg (M); **LOEL** = 5 mg/kg (both sexes).

Brain ChE NOEL = 0.5 mg/kg (F) and < 5 mg/kg (M); **LOEL** = 2.5 mg/kg (F) and < 5.0 mg/kg (M).

Considering the findings in this range-finding study and those in the earlier range-finding study (MRID 44203301), doses of 10, 100 and 500 mg/kg and a time peak of approximately 2.5 hours after dosing (day 0) were selected for the main acute neurotoxicity study with ORTHENE® Technical (81-8; MRID 44203303). However, considering the ChE inhibition data in this range-finging study, the 10 mg/kg dose appears to be too high for the lowest dose in the acute neurotoxicity study.

012416

MEMORANDUM:

SUBJECT:

EPA ID NOs: DP Barcode: D233251
Submission No.:
PC Code: 103301
MRID Nos.: 442033-04

FROM: Kathleen C. Raffaele Kathleen C Raffael

TO:

THRU: Steve Dapson
Branch Senior Scientist
Toxicology Branch II
Health Effects Division (7509C)

REGISTRANT: Valent U.S. A. Corporation

CHEMICAL: ORTHENE Technical (Acephate)

ACTION REQUESTED: Review Subchronic Neurotoxicity study

EXECUTIVE SUMMARY: Nemec, M.D., (1997); A Subchronic (13-week) Neurotoxicity Study of ORTHENE Technical in Rats, WIL Research Laboratories, Inc., Ashland, Ohio, Lab Project Identification WIL-194014, 1/16/97, MRID No.:44203304; unpublished.

Acephate (99% purity) was administered to Sprague Dawley rats (30/sex/group) at 0, 5, 50, or 700 ppm in the diet (mean compound intake was 0.33, 3.31, and 48.63 mg/kg/day for males, 0.41, 3.95, and 58.27 mg/kg/day for females, respectively) for 13 weeks. Body weights were recorded weekly, food consumption was recorded twice weekly, and clinical observations were recorded daily. Cholinesterase activity was determined in plasma, erythrocytes, and brain (6 regions) at weeks 3, 7, and at study termination in 6 animals/sex/group. Neurobehavioral assessment (functional observation battery and motor activity testing) was performed in 12 animals/sex/group prior to compound administration and during study weeks 3, 7, and 12. Brain weights (whole brain and regional) were determined during study weeks 3, 7, and at study termination in non-perfused animals (6/sex/group). At study termination, 12 animals/sex/group were euthanized and perfused in situ for neuropathological examination; brain weights and measures were determined. Of the perfused animals, 5/sex for control and 700 ppm groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

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The only effects seen at the 5 ppm dose were inhibition of brain cholinesterase (significant in at least one sex for all brain regions, inhibition ranged from 2 to 28%).

At 50 ppm dose, there was significant inhibition of brain cholinesterase in all regions for both sexes (ranging from 18-55%). Plasma cholinesterase was inhibited at 50 ppm for males and females at week 3 (25-41%). Erythrocyte cholinesterase was not significantly inhibited, but was decreased by 26% in females at week 3. Thus, the NOEL for plasma cholinesterase inhibition was 5 ppm, with a LOEL of 50 ppm. Other effects seen at 50 ppm included a slight increase in clinical signs, specifically hair loss.

At the 700 ppm dose, brain and plasma cholinesterase were significantly inhibited in both sexes at all time points (range 55-74% inhibition for plasma, 63-82% inhibition for brain). Erythrocyte cholinesterase was significantly inhibited in both sexes at all time points (37-46%) except for week 13 females (25% inhibition). Thus, the NOEL for erythrocyte cholinesterase was 50 ppm, with a LOEL of 700 ppm. Additional effects seen at 700 ppm included decreased body weight (males) and body weight gain (males and females); increased food consumption (when measured as g/kg/day); increased grooming, increased rearing, and decreased rotarod time in males; decreased motor activity in females.

Based on the effects seen in this study, the LOEL for systemic effects (increases in clinical signs) was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for neurotoxicity (FOB findings and decreased motor activity) was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for erythrocyte cholinesterase inhibition was 700 ppm (48.63 or 58.27 mg/kg/day for males or females, respectively), with a NOEL of 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively). The LOEL for plasma cholinesterase inhibition was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for brain cholinesterase inhibition was 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively), with the NOEL less than 5 ppm (the lowest dose tested).

The study is classified as acceptable for subchronic neurotoxicity in rats.

cc:

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Reviewed by: Kathleen C. Raffaele, Ph.D. Kathleen C. Raffaele
Tox Branch II (7509C)

Secondary Reviewer: John Doherty, Ph.D. Stephen C. Kaplan 11/4/97
Tox Branch II (7509C)

DATA EVALUATION RECORD

012416

STUDY TYPE: Subchronic Neurotoxicity

EPA ID NOs: MRID No.: 442033-04
Pesticide Chemical Code: 103301
Toxicology Chemical Code:
DP Barcode: D233251
Submission No.:
CAS Reg. No.: 30560-19-1

TEST MATERIAL: ORTHENE Technical (Acephate)

CITATION: Nemec, M.D., (1997); A Subchronic (13-week) Neurotoxicity Study of ORTHENE Technical in Rats, WIL Research Laboratories, Inc., Ashland, Ohio, Lab Project Identification WIL-194014, 1/16/97, MRID No.:44203304; unpublished.

SPONSOR: Valent U.S.A. Corporation, Walnut Creek, CA

EXECUTIVE SUMMARY: Acephate (99% purity) was administered to Sprague Dawley rats (30/sex/group) at 0, 5, 50, or 700 ppm in the diet (mean compound intake was 0.33, 3.31, and 48.63 mg/kg/day for males, 0.41, 3.95, and 58.27 mg/kg/day for females, respectively) for 13 weeks. Body weights were recorded weekly, food consumption was recorded twice weekly, and clinical observations were recorded daily. Cholinesterase activity was determined in plasma, erythrocytes, and brain (6 regions) at weeks 3, 7, and at study termination in 6 animals/sex/group. Neurobehavioral assessment (functional observation battery and motor activity testing) was performed in 12 animals/sex/group prior to compound administration and during study weeks 3, 7, and 12. Brain weights (whole brain and regional) were determined during study weeks 3, 7, and at study termination in non-perfused animals (6/sex/group). At study termination, 12 animals/sex/group were euthanized and perfused in situ for neuropathological examination; brain weights and measures were determined. Of the perfused animals, 5/sex for control and 700 ppm groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

The only effects seen at the 5 ppm dose were inhibition of brain cholinesterase (significant in at least one sex for all brain regions, inhibition ranged from 2 to 28%).

At 50 ppm dose, there was significant inhibition of brain cholinesterase in all regions for both sexes (ranging from 18-55%).

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Plasma cholinesterase was inhibited at 50 ppm for males and females at week 3 (25-41%). Erythrocyte cholinesterase was not significantly inhibited, but was decreased by 26% in females at week 3. Thus, the NOEL for plasma cholinesterase inhibition was 5 ppm, with a LOEL of 50 ppm. Other effects seen at 50 ppm included a slight increase in clinical signs, specifically hair loss.

At the 700 ppm dose, brain and plasma cholinesterase were significantly inhibited in both sexes at all time points (range 55-74% inhibition for plasma, 63-82% inhibition for brain). Erythrocyte cholinesterase was significantly inhibited in both sexes at all time points (37-46%) except for week 13 females (25% inhibition). Thus, the NOEL for erythrocyte cholinesterase was 50 ppm, with a LOEL of 700 ppm. Additional effects seen at 700 ppm included decreased body weight (males) and body weight gain (males and females); increased food consumption (when measured as g/kg/day); increased grooming, increased rearing, and decreased rotarod time in males; decreased motor activity in females.

Based on the effects seen in this study, the LOEL for systemic effects (increases in clinical signs) was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for neurotoxicity (FOB findings and decreased motor activity) was 700 ppm (48.63 or 58.27 mg/kg/day for males or females, respectively), with a NOEL of 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively). The LOEL for erythrocyte cholinesterase inhibition was 700 ppm (48.63 or 58.27 mg/kg/day for males or females, respectively), with a NOEL of 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively). The LOEL for plasma cholinesterase inhibition was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for brain cholinesterase inhibition was 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively), with the NOEL less than 5 ppm (the lowest dose tested).

The study is classified as acceptable for subchronic neurotoxicity in rats.

COMPLIANCES: GLP statement, p. 3, QA statement p. 46.

I. MATERIALS

A. Test Compound: ORTHENE Technical (Acephate, O,S-Dimethyl acetylphosphoramidothioate); Description: White powder; Lot No: SX1725 (expiration date 6/13/96); Purity: 99.0%; Contaminants: not stated.

B. Test Animals:
Species: Rat
Strain: Sprague-Dawley, Crl:CDBR
Age: 51 days at dosing initiation
Acclimation: 26 days prior to dosing initiation (including one-week pre-test period)
Weight at initiation (g): (Males 208-316 g, Females 138-213 g)
Source: Charles River Laboratories, Portage, Mich.
Housing: Individual, in suspended wire-mesh cages
Feed: Purina Certified Rodent Chow #5002, ad lib.
Water: municipal water
Environmental: 12/12 light/dark cycle, temperature range 72-76° F, humidity 37-63.5%
In-life dates: 2/6/96 to 5/10/96

II. METHODS

A. **Study Design:** Groups consisted of 30 male and 30 female rats. Animals were randomly assigned to groups, stratified for body weight. Test substance was administered in the diet at levels of 0, 5, 50, or 700 ppm for up to 13 weeks. Dose levels were chosen based on the subchronic dietary study in rats (MRID 40504819, 1987) and the lifetime oral toxicity/carcinogenicity study in rats (MRID 00084017, 1981) [findings from those studies included decreased body weights in males and 66-83% reduction in brain cholinesterase for rats exposed to 700 ppm acephate in the diet].

Eighteen rats/sex/group were assigned to cholinesterase determination (6/sex/group were sacrificed at 3, 7, and 13 weeks for determination of plasma, red blood cell, and brain region cholinesterase); whole brain weight and regional brain weights were also determined for these animals at sacrifice. The other 12 animals/sex/group received Functional Observation Battery (FOB) and Locomotor Activity assessments prestudy and during study weeks 3, 7, and 12 (see Table A). Behavioral testing was conducted as 4 study replicates on 4 consecutive days (because of the large number of animals to be tested); test groups were balanced across study replicates. At study termination, neuropathological evaluations were performed on those animals from the behavioral testing groups.

Table A. Study Design

Experimental Parameter	Dose Group ppm (mg/kg/day)			
	0 ppm	5 ppm (0.33)	50 ppm (3.31)	700 ppm (48.63)
Total number of Animals/sex/group	30	30	30	30
Blood and brain cholinesterase determinations - week 3	6	6	6	6
Blood and brain cholinesterase determinations - week 7	6	6	6	6
Blood and brain cholinesterase determinations - week 13	6	6	6	6
Behavioral testing and Neuropathology	12	12	12	12

B. **Diet Preparation**: Appropriate amounts of test substance were dissolved in acetone; the solutions were then mixed with diet to achieve desired dose levels. Control diet was mixed with acetone only. After completion of mixing, diet was placed in storage bags and left open overnight to allow evaporation of acetone. Prepared diet was stored frozen, and initially dispensed daily. After verification of 4-day stability at room temperature, diets were dispensed twice weekly. Fresh diet was prepared weekly. Diets were analyzed for homogeneity and stability during week 0 and for concentration during weeks 0, 1, 2, 3, 7, and 11.

C. **Observations**

1. Mortality and clinical observations: Animals were observed twice daily for mortality and moribundity. Detailed clinical observations were recorded daily (except on the day of FOB administration).

2. Body weights: Body weights were recorded weekly, on days when the FOB was administered, and at study termination.

3. Food consumption: Food consumption was recorded daily for the first two weeks of study and twice weekly thereafter; the data is presented for weekly intervals (corresponding to the body weight intervals). Test article consumption was calculated by sex and dose group based on mean food consumption (g/kg/day) and test substance concentration.

D. Cholinesterase Determination: Cholinesterase activity was determined in 6 animals/sex/group during study weeks 3 and 7 and at termination. Non-fasted animals were sacrificed (using carbon dioxide) and blood collected from the inferior vena cava; brain was excised from the skull, weighed, and dissected (weight was recorded for whole brain and for brain regions). Brain cholinesterase was determined for olfactory region, cerebral cortex, cerebellum, brain

stem, hippocampus, and midbrain (including striatum). 'True red blood cell' cholinesterase activity was derived from values for whole blood cholinesterase, plasma cholinesterase, and hematocrit (see equation below, from p. 29, vol. 1 of study report). Cholinesterase activities were measured using a modification of the Ellman method (with 6,6'-dithiodinicotinic acid, with a primary wavelength of 340 nm), using a Hitachi 911 chemistry analyzer.

$$RBC = \frac{(\text{Whole blood ChE value} \times 10) - [\text{Plasma ChE value} \times (1 - \text{Hematocrit})]}{\text{Hematocrit}}$$

Hematocrit is expressed as decimal equivalent; x10 compensates for dilution factor during hemolysis procedure.

E. **Neurobehavioral Assessment:**

1. Functional Observational Battery (FOB): Testing was performed by the same individuals throughout the study (to the extent possible); technicians were blind to the individual animal's treatment group. Except for home cage observations (performed in the animal room), testing was performed in a sound-proof room with a white noise generator operating at 70 db. Scoring criteria are described in Appendix D (vol. 4, p. 1123). The following parameters were evaluated:

Home cage observations: posture, biting, convulsions/tremors, palpebral (eyelid) closure, feces consistency;
Handling observations: ease of removal from cage, ease of handling animal in hand, lacrimation/chromodacryorrhea, salivation, piloerection, fur appearance, palpebral closure, respiratory rate/character, red/crusty deposits, mucous membranes/eye/skin color, eye prominence, muscle tone;
Open field observations (2 minute observation period): mobility, gait, rearing, arousal, convulsions/tremors, urination/defecation, grooming, gait score, bizarre/stereotypic behavior, backing, time to first step (seconds);
Sensory observations: approach response, touch response, startle response, tail pinch response, pupil response, eyeblink response, forelimb extension, hindlimb extension, air righting reflex, olfactory orientation;
Neuromuscular observations: hindlimb extensor strength, grip strength-hind and forelimb, hindlimb foot splay, rotarod performance;
Physiological observations: catalepsy, body weight, body temperature.

2. Locomotor Activity: Locomotor Activity was evaluated after completion of the FOB. Testing was done in replicate sequence. Data were recorded automatically, using the Digiscan 'Micro' Animal Activity System (Omnitech Electronics, Inc., Columbus, OH), over a forty-one minute period. Data are reported for the entire 40 minute period and for four 10-minute subsessions (data from the first minute were discarded due to incomplete data

collection), as total activity (interruption of one or two adjacent photobeams) and ambulatory activity (interruption of 3 or more consecutive photobeams).

F. **Sacrifice and Pathology:** 12 animals/sex/group were sacrificed at week 13, via carbon dioxide inhalation, and perfused for neuropathologic examination. Brain weight (excluding olfactory bulbs) and dimensions were recorded, and tissues were prepared for histopathological examination. For five animals/sex from the control and 700 ppm groups, the following tissues were examined histopathologically. Central nervous system (embedded in paraffin): brain (forebrain, center of cerebrum, midbrain, cerebellum and pons, medulla oblongata), spinal cord (at cervical and lumbar swellings), gasserian ganglion/trigeminal nerves, lumbar dorsal root ganglion and fibers, lumbral ventral root fibers, cervical dorsal root ganglion and fibers, cervical ventral root fibers, optic nerves, and eyes. Peripheral nervous system (embedded in plastic): sciatic nerve (mid-thigh region and at sciatic notch), sural nerve, tibial nerve, peroneal nerve. Forelimbs and tail were also preserved but not examined.

G. **Positive and Historical Controls:** Summarized data from several positive control validation studies (dated 1990 and 1991), and an inter-observer reliability study (dated 1991) were included as Appendix E (Vol. 4, pp. 1138-1168). Historical control data were also submitted (Appendix H, Vol. 5, pp. 1498-1552 for FOB data; Appendix I, Vol. 5, pp. 1553-1557 for motor activity; Appendix J, Vol. 5, pp. 1558-1601 for neuropathology). Performance dates for the studies included in the historical control data were not submitted.

H. **Statistical Evaluations:** Continuous data (except for locomotor activity data) were analyzed statistically using one-way analysis of variance and Dunnett's test for comparison of control and treated groups. Discontinuous FOB data were analyzed using Fisher's Exact Test. Locomotor activity data were analyzed using two-way repeated measures analysis of variance. Significant findings were followed by one-way analysis of variance for each time point, and Dunnett's multiple-T test (see Vol. 1, p. 31). Significance was determined at the 5% and 1% levels.

IV. RESULTS

A. **Analytical Chemistry:** The product analysis sheet certified product purity at 99% a.i. on 6/13/95 (p. 1098, Vol.4). Homogeneity analysis demonstrated homogeneity of diet mixtures when acetone was used as the dissolving solvent (all samples were within 5% of target concentration, p. 1109, v. 4). Stability was demonstrated following 8 days of frozen storage (92.1%, 98.1%, and 105% of day zero concentrations for 5 ppm, 50 ppm, and 700 ppm dose formulations, respectively), and 16 days of frozen storage (108% of day zero concentrations for all dose formulations). After 4 days

of room temperature storage, stability was somewhat less (89.5%, 89.9%, and 87.8% of day zero for 5 ppm, 50 ppm, and 700 ppm dose formulations, respectively), but was considered acceptable. Stability was considered unacceptable after 14 days of room temperature storage (74.3%, 70.9%, and 68.6% of day zero concentration for 5 ppm, 50 ppm, and 700 ppm, respectively). Concentration analyses for subsequent study weeks were all within 10% of target concentrations.

B. **Clinical signs and mortality:** All animals survived to study termination. Study authors stated that there were no treatment-related increases in clinical signs. There were, however, slight dose-related increases in the incidence of hair loss in mid- and high-dose males and females, an increase in 'dried red material around prepuce' in treated males, and an increase in incidence of red and swollen ears in treated females (see Table I).

Table I: Clinical observations.

Observation	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Males				
Hair Loss				
- left forelimb	31/1	8/1	205/5	327/6
- right forelimb	32/1	1/1	222/5	329/5
- base of tail	15/1	0	0	87/5
Dried red material around prepuce	1/1	1/1	3/2	7/6
Females				
Hair loss				
- left forelimb	7/2	29/2	96/3	88/8
- right forelimb	4/3	33/3	99/3	137/8
- base of tail	0/0	0/0	77/2	15/3
Ear appears red and swollen				
- right ear	124/8	387/13	351/13	238/16
- left ear	88/5	300/11	244/13	128/12

Data were extracted from Table 1, vol. 1, pp. 49-55. Numbers represent the total number of observations/number of animals with at least one instance of the observation.

C. **Body weight and body weight gain:** Body weight in high dose males was slightly less than that of control males, significant only during weeks 1-3. There were no other significant differences in body weights among treated males or females, at any

time point during the study. Body weight gain was significantly decreased in high dose males and females during week 0-1 only (see Table II). There was a significant increase in body weight gain for low dose males during study week 1-2, but as this effect did not occur in the other treatment groups at this time point, and was not dose-related; it does not appear to be compound-related. Note that fewer animals are included in the mean values at later time points, due to interim sacrifice of cholinesterase animals.

Table II. Body weight and body weight gain (grams).

Observation	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Body weight-Males				
-Week 0	269 (16.6)	270 (20.3)	266 (17.8)	265 (25.3)
-Week 1	321 (25.9)	317 (21.2)	314 (20.5)	301** (27.2)
-Week 2	357 (29.5)	361 (23.8)	352 (23.6)	337* (31.7)
-Week 3	387 (33.3)	392 (25.0)	384 (25.2)	364* (32.5)
-Week 4	413 (35.6)	420 (27.0)	413 (30.0)	391 (35.5)
-Week 13	533 (50.5)	562 (32.0)	541 (40.2)	511 (44.2)
Body weight-Females				
-Week 0	173 (10.8)	169 (18.2)	174 (14.6)	173 (12.8)
-Week 1	195 (13.4)	191 (19.9)	196 (14.1)	190 (13.1)
-Week 2	214 (15.4)	206 (22.1)	215 (17.6)	208 (16.0)
-Week 13	295 (28.8)	288 (43.7)	301 (35.0)	300 (32.7)
Body weight gain-Males				
-Week 0-1	52 (8.4)	47 (10.2)	48 (9.0)	36** (5.8)
-Week 1-2	36 (7.1)	44** (9.6)	38 (6.2)	36 (7.4)
Body weight gain-Females				
-Week 0-1	23 (8.0)	22 (5.4)	22 (4.8)	18** (6.9)
-Week 1-2	18 (5.5)	15 (6.5)	19 (6.4)	18 (6.0)

Data were extracted from Tables 2 and 3, Vol. 1, pp. 56-69. Values represent mean (s.d.); n=30 for weeks 1-3, n=24 for week 4, n=18 for week 13. *=p<.05, **=p<.01, when compared to control means.

D. **Food consumption and achieved compound intake:** There was no difference in food consumption, when measured as g/animal/day, at any time point for males or females. However, there was a significant increase in food consumption, when measured as g/kg/day, for both high dose males (weeks 1-2 through 7-8) and females (weeks 1-2 through 4-5) (see Table III).

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Table III. Food consumption (g/kg/day).

Week No.	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Males				
0-1	96 (5.5)	95 (5.7)	96 (5.4)	96 (5.9)
1-2	84 (5.3)	88* (5.1)	86 (4.6)	89** (4.2)
2-3	78 (4.6)	78 (4.3)	79 (3.6)	82** (4.4)
4-5	67 (4.1)	69 (3.7)	69 (3.2)	73** (3.7)
7-8	60 (4.1)	59 (3.6)	59 (3.2)	64** (5.1)
Females				
0-1	103 (6.6)	103 (7.3)	102 (8.5)	103 (7.0)
1-2	96 (6.1)	98 (6.3)	98 (6.5)	102** (7.4)
2-3	94 (6.0)	95 (5.4)	92 (7.5)	101** (6.5)
4-5	82 (4.4)	85 (9.1)	83 (6.3)	88** (6.7)

Data were extracted from Table 5, Vol. 1, pp. 76-81. Values represent mean (s.d.); n=30 for weeks 0-1 through 2-3, n=24 for weeks 3-4 through 6-7, n=18 for weeks 7-8 through 12-13. ** significantly different from control means at p<.01.

Mean test article consumption was 0.33, 3.31, and 48.63 for males at 5, 50, and 700 ppm groups, respectively; for females, test article consumption was 0.41, 3.95, and 58.27 mg/kg/day for 5, 50, and 700 ppm groups, respectively.

E. **Cholinesterase activities:** Plasma cholinesterase was significantly inhibited for both male and females in the 50 ppm group (week 3 only) and the 700 ppm group (all timepoints). There was also a nonsignificant 22% inhibition of plasma cholinesterase in 5 ppm females at week 3. RBC cholinesterase was significantly inhibited for males and females in the 700 ppm group only (see Table IV). There was a very large variance in blood cholinesterase values (especially in the females), which may account for the lack of statistical significance for some groups and time points where large percent inhibition was seen (for example, 26% inhibition found in 'true RBC cholinesterase' in females at week 3).

Table IV. Blood Cholinesterase Activity.

Observation	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Plasma ChE (U/l)				
Males				
Week 3	739 (95.0)	706 (106.4) [-4]	552* (123.7) [-25]	319** (64.7) [-57]
Week 7	647 (109.3)	624 (98.9) [-4]	576 (93.8) [-11]	291** (53.3) [-55]
Week 13	733 (126.5)	862 (205.9) [+18]	662 (75.5) [-10]	328** (30.6) [-55]
Females				
Week 3	3050 (743.2)	2386 (863.6) [-22]	1798** (569.2) [-41]	782** (302.4) [-74]
Week 7	3353 (1181.2)	3348 (1332.6) [<-1]	2628 (1155.5) [-22]	917** (171.1) [-73]
Week 13	3409 (1271.4)	3950 (1170.6) [+16]	3211 (943.9) [-6]	1137** (416.9) [-67]
True RBC ChE (U/L)				
Males				
Week 3	3815 (594.1)	3145 (475.4) [-18]	3302 (748.8) [-13]	2070** (356.3) [-46]
Week 7	3491 (525.4)	3521 (193.2) [+1]	3215 (346.3) [-8]	2207** (94.7) [-37]
Week 13	3562 (363.4)	3548 (321.8) [<-1]	3171 (580.9) [-11]	2257** (212.5) [-37]
Females				
Week 3	3139 (833.6)	2903 (1070.2) [-8]	2316 (816.4) [-26]	1814* (458.1) [-42]
Week 7	3238 (269.2)	3363 (609.3) [+4]	2983 (391.4) [-8]	1857** (360.2) [-43]
Week 13	3077 (941.4)	3380 (691.8) [+10]	2789 (1063.0) [-9]	2309 (198.3) [-25]

Data were extracted from Table 55, Vol. 1, pp. 256-257 and p. 38. Values represent mean (s.d.) [% difference from control mean]; **=p<.01, *=p<.05, when compared to control mean. N=6, except for females, week 13, 50 ppm, plasma and RBC ChE (n=5), and females, week 7, 5 ppm RBC ChE (n=5).

Brain cholinesterase was inhibited significantly for both males and females at all dose levels, at varying time points for varying brain regions (see Table V).

Table V. Brain Cholinesterase Activity (u/g).

Brain region	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Hippocampus				
Male				
Week 3	9.00 (0.908)	7.75* (0.311) [-14]	5.57* (0.893) [-38]	1.90** (0.164) [-79]
Week 7	9.46 (0.719)	8.76 (1.439) [-7]	4.99** (0.300) [-47]	1.73** (0.101) [-82]
Week 13	9.66 (1.354)	8.13 (1.124) [-16]	6.84 (4.441) [-29]	1.87** (0.190) [-81]
Female				
Week 3	9.32 (4.281)	8.76 (2.511) [-6]	4.86* (0.509) [-48]	2.06** (1.189) [-78]
Week 7	9.29 (1.082)	8.85 (1.171) [-5]	4.66** (0.271) [-50]	1.71** (0.176) [-82]
Week 13	10.46 (1.238)	7.49** (0.546) [-28]	4.75** (0.387) [-55]	1.90** (0.160) [-82]
Olfactory				
Male				
Week 3	16.54 (2.515)	15.74 (3.486) [-5]	9.14** (2.946) [-45]	3.76** (0.824) [-77]
Week 7	16.94 (1.693)	14.94 (5.677) [-12]	10.09** (2.276) [-40]	3.09** (0.460) [-82]
Week 13	17.40 (4.219)	16.55 (3.784) [-5]	10.65** (2.741) [-39]	3.68** (0.388) [-79]
Female				
Week 3	13.84 (4.848)	15.70 (1.109) [+13]	10.58 (0.945) [-24]	2.93** (0.994) [-79]
Week 7	19.94 (5.183)	15.09* (0.850) [-24]	9.13** (1.309) [-54]	3.51** (0.465) [-82]
Week 13	17.93 (2.498)	14.74* (1.898) [-18]	9.84** (2.417) [-45]	3.83** (0.243) [-79]

Midbrain				
Male				
Week 3	14.68 (0.938)	13.29** (0.304) [-9]	9.45** (0.780) [-36]	3.92** (0.323) [-73]
Week 7	15.79 (1.338)	13.55** (0.677) [-14]	9.54** (0.951) [-40]	3.61** (0.146) [-77]
Week 13	15.56 (1.020)	13.30** (1.904) [-15]	9.59** (0.903) [-38]	3.79** (0.252) [-76]
Female				
Week 3	17.00 (2.898)	13.75** (1.000) [-19]	9.41** (0.406) [-45]	3.95** (0.565) [-77]
Week 7	16.49 (2.741)	13.97* (0.936) [-15]	8.76** (0.348) [-47]	3.59** (0.179) [-78]
Week 13	14.65 (0.712)	13.33* (0.843) [-9]	9.50** (0.805) [-35]	3.96** (0.358) [-73]
Brainstem				
Male				
Week 3	13.15 (0.806)	11.18** (0.529) [-15]	9.09** (0.635) [-31]	4.20** (0.260) [-68]
Week 7	12.96 (0.844)	11.74** (0.589) [-9]	8.57** (0.676) [-34]	3.77** (0.307) [-71]
Week 13	12.06 (0.809)	10.80** (0.426) [-10]	8.77** (0.531) [-27]	3.85** (0.383) [-68]
Female				
Week 3	13.04 (0.475)	12.16 (0.856) [-7]	10.63 (3.539) [-18]	4.13** (0.373) [-68]
Week 7	14.09 (1.748)	12.91 (0.718) [-8]	9.08** (0.452) [-36]	3.96** (0.175) [-72]
Week 13	12.33 (1.363)	11.81 (0.563) [-4]	8.59** (0.465) [-30]	3.82** (0.195) [-69]

Cerebellum				
Male				
Week 3	5.85 (0.426)	5.21** (0.254) [-11]	4.29** (0.238) [-27]	2.19** (0.139) [-63]
Week 7	5.71 (0.283)	5.51 (0.351) [-4]	4.13** (0.265) [-28]	1.99** (0.134) [-65]
Week 13	5.65 (0.365)	5.34 (0.514) [-5]	4.28** (0.375) [-24]	2.09** (0.256) [-63]
Female				
Week 3	5.84 (0.323)	5.68 (0.561) [-3]	4.28** (0.608) [-27]	2.08** (0.181) [-64]
Week 7	6.70 (0.939)	5.62** (0.399) [-16]	4.18** (0.212) [-38]	2.10** (0.243) [-69]
Week 13	5.65 (0.439)	5.41 (0.307) [-4]	4.26** (0.233) [-25]	2.09** (0.069) [-63]
Cortex				
Male				
Week 3	14.65 (1.108)	12.29** (0.876) [-16]	8.26** (0.356) [-44]	2.90** (0.306) [-80]
Week 7	14.44 (0.770)	12.98** (1.049) [-10]	7.83** (0.619) [-46]	2.60** (0.136) [-82]
Week 13	14.96 (1.595)	12.33** (1.004) [-18]	7.57** (1.375) [-49]	2.93** (0.678) [-80]
Female				
Week 3	13.26 (1.413)	12.37 (1.176) [-7]	8.11** (0.408) [-39]	2.51** (0.307) [-81]
Week 7	13.89 (2.224)	13.56 (0.928) [-2]	7.77** (0.664) [-44]	2.67** (0.168) [-81]
Week 13	14.81 (1.129)	12.76** (0.833) [-14]	7.29** (0.563) [-51]	2.82** (0.351) [-81]

Data were extracted from Table 56, Vol. 1, pp. 258-263 and p. 38. Values represent mean (s.d.) [% difference from control mean]; **=p<.01, *=p<.05, when compared to control mean. N=6 for all data points.

Although the study author asserted that both changes in plasma cholinesterase, and significant inhibition of brain cholinesterase at levels less than 20% were not toxicologically relevant, this is inconsistent with Agency policy. Therefore, the significant brain cholinesterase inhibition is considered to be toxicologically relevant. In addition, inhibition of large magnitude, even if not statistically significant, can be considered toxicologically relevant in some instances (for example if the lack of significance is due to extremely large variance).

F. **Neurobehavioral results**

1. FOB Findings: There were no differences among treatment groups, for males or females, with regard to home cage observations, handling observations, or sensory observations at any time point.

For the physiological observations, the only significant difference was a decrease in catalepsy time for 50 ppm and 700 ppm females during week 7 (times were 0.6 sec, 0.5 sec, 0.5 sec, and 0.4 sec for control, 5 ppm, 50 ppm, and 700 ppm groups, respectively). There were no significant effects in males. The differences were small in magnitude and are not considered toxicologically relevant.

There were no significant differences among female groups for open field observations. For males, there were significant increases in rearing and grooming for 700 ppm group during week 3 (see Table VI). These differences appear to be dose-related (there is a non-significant increase in the 50 ppm group for both measures at the same time point), and are outside the historical control range (Vol. 5, pp. 1513, 1504). However, they are small in magnitude and do not persist throughout the treatment period, therefore their toxicological relevance is questionable.

The only significant differences found for neuromuscular observations were for hind limb splay and rotarod times (males only; see Table VI). The differences in hind limb splay are not dose- or time-dependent, and thus are not considered treatment-related. Although the decrease in rotarod performance was significant only at week 7, it persisted at week 12, and appears to be below the historical control data (mean time for 40 animals in 4 studies, week 12, was 67.6 $\pm$ 7.9 sec [v. 5, p. 1516]; no range was provided). Therefore, the decrease in rotarod performance may be treatment related. There were no significant changes in rotarod times for treated females.

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Table VI. Functional Observation Battery results.

Observation	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Open field observations				
Males				
Grooming-wk. 3	0.2 (0.58)	0.2 (0.39)	0.4 (0.90)	0.9* (0.90)
Grooming-wk. 7	0.1 (0.29)	0.2 (0.39)	0.0 (0.0)	0.7 (1.15)
Rearing - wk. 3	7.9 (4.36)	7.3 (3.79)	9.0 (4.16)	12.1* (4.10)
Rearing - wk. 7	8.4 (2.91)	8.7 (4.52)	11.0 (5.92)	9.1 (5.48)
Neuromuscular observations				
Males				
Hindlimb splay wk. 3	82.0 (14.42)	69.9 (16.18)	61.8** (11.91)	80.7 (16.69)
Hindlimb splay wk.7	66.0 (17.04)	51.4 (15.38)	52.0 (14.35)	62.9 (18.10)
Hindlimb splay wk.12	74.7 (15.90)	55.0** (17.88)	48.2** (10.83)	61.3 (16.38)
Rotarod time wk. 3	57.2 (48.08)	44.6 (36.19)	90.8 (45.05)	35.5 (25.37)
Rotarod time wk. 7	73.7 (55.25)	56.9 (46.00)	62.7 (51.40)	21.9* (23.48)
Rotarod time wk.12	47.7 (47.89)	62.8 (51.86)	65.7 (45.87)	27.6 (37.02)

Data were extracted from Tables 25 (Vol. 1,p. 161) and 28 (Vol. 1, p. 173) of the study report. Grooming and rearing are measured as counts/2 min. period, hindlimb splay is measured in mm, rotarod time is in sec. Values in parentheses are standard deviations. n=12 for all time points. *=p<.05,** p<.01 compared with controls.

2. Motor activity: There were no differences among groups for motor activity in males. There was a significant decrease in total activity and ambulatory activity (grand total and subsession 3) in 700 ppm females at week 7, and a significant decrease in subsession 4 ambulatory activity for females in the 700 ppm group, week 12 (see Table VII). These effects are seen in the 700 ppm group only, with no dose-related trend in the lower groups. There does appear to be consistency across time, however, with the effect occurring at both 7 and 12 weeks. Thus, although this effect is considered marginal, it is possibly compound-related.

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Table VII. Motor activity Counts - Females.

Subsession #	Dose Group			
	0 ppm	5 ppm	50 ppm	700 ppm
Total Activity Week 7				
Subsession 3	385 (184.1)	298 (107.9)	412 (135.0)	234* (38.4) [-39%]
Subsession 4	339 (131.0)	225 (168.6)	300 (132.6)	203 (127.2) [-40%]
Grand Total	1903 (378.9)	1714 (465.3)	2051 (488.9)	1454* (253.3) [-24%]
Ambulatory Activity Week 7				
Subsession 3	252 (118.5)	209 (68.1)	285 (84.4)	157* (27.2) [-38%]
Subsession 4	231 (82.8)	160 (124.9)	199 (84.9)	146 (91.8) [-37%]
Grand Total	1256 (250.3)	1142 (269.4)	1364 (270.1)	987* (159.6) [-21%]
Ambulatory Activity Week 12				
Subsession 3	224 (124.8)	144 (113.3)	186 (110.9)	147 (76.3) [-34%]
Subsession 4	168 (113.3)	103 (61.0)	147 (100.3)	66* (50.2) [-61%]
Grand Total	1122 (418.6)	919 (336.8)	1102 (331.2)	778 (181.5) [-31%]

Data were extracted from Tables 53 (pp. 250-251) 54 (p. 255). Grand total value is the sum of subsessions 1-4. Values represent mean (s.d.) [percent change from control], n=12 for all time points. * = p<.05 when compared with control.

G. Sacrifice and pathology:

1. Gross pathology: For non-perfused animals, there were scattered significant differences in absolute brain weights, brain/body weight ratios, and brain region/whole brain weight ratios. There was no evidence of dose or time-dependence for any of these findings, and they were not considered treatment related. For perfused animals (week 13), there were no significant differences for brain weight, length or width for males. For females, brain length was increased in a dose-related fashion, significant at 700 ppm ($p<.05$; values were 19.7, 20.2, 20.3, and 20.5* mm for 0, 5, 50, and 700 ppm groups, respectively). Brain width was also increased in treated females, significant at 50 ppm dose only ($p<.05$; values were 14.2, 14.6, 14.8*, and 14.7 for 0, 5, 50, and 700 ppm groups, respectively). No historical control data was submitted for brain measures. In the absence of

histopathology, changes in brain measures of this magnitude are not considered toxicologically relevant.

2. Neuropathology: Scattered lesions were seen in control and high dose animals, including axonal degeneration and digestion chambers in lumbar dorsal and ventral fibers and in peripheral nerves. These changes were found with similar incidence and severity for both groups, and do not appear to be treatment related.

V. DISCUSSION and CONCLUSIONS:

Acephate (99% purity) was administered to Sprague Dawley rats (30/sex/group) at 0, 5, 50, and 700 ppm in the diet (mean compound intake was 0.33, 3.31, and 48.63 mg/kg/day for males, 0.41, 3.95, and 58.27 mg/kg/day for females, respectively) for 13 weeks. The study is considered well reported, and historical and positive control data were supplied (with the exception of historical controls for brain measurements).

There was a very large variance in results for the blood cholinesterase assays, especially for females. It is possible that, had a less variable assay been available, the significant inhibition of plasma and erythrocyte cholinesterase inhibition would have been demonstrated at lower dose levels, resulting in lower NOELs for cholinesterase inhibition. Study authors, in fact, found the LOEL for plasma cholinesterase to be 5 ppm for females, and the LOEL for erythrocyte cholinesterase inhibition to be 50 ppm for females. Due to the large variability across time points and sexes at these dose levels, this reviewer did not feel these doses could be considered effect levels.

The only effects seen at the 5 ppm dose were inhibition of brain cholinesterase (significant in at least one sex for all brain regions, inhibition ranged from 2 to 28%). Although the study author discounted significant increases in cholinesterase inhibition as biologically irrelevant when the change was less than 20%, this is inconsistent with current Agency policy. Thus the NOEL for brain cholinesterase inhibition is less than 5 ppm, with a LOEL of 5 ppm.

At 50 ppm dose, there was significant inhibition of brain cholinesterase in all regions for both sexes (ranging from 18-55%). Plasma cholinesterase was inhibited at 50 ppm for males and females at week 3 (25-41%). Erythrocyte cholinesterase was not significantly inhibited, but was decreased by 26% in females at week 3. Thus, the NOEL for plasma cholinesterase inhibition was 5 ppm, with a LOEL of 50 ppm. Other effects seen at 50 ppm included a slight increase in clinical signs, specifically hair loss.

At the 700 ppm dose, brain and plasma cholinesterase were

significantly inhibited in both sexes at all time points (range 55-74% inhibition for plasma, 63-82% inhibition for brain). Erythrocyte cholinesterase was significantly inhibited in both sexes at all time points (37-46%) except for week 13 females (25% inhibition). Thus, the NOEL for erythrocyte cholinesterase was 50 ppm, with a LOEL of 700 ppm. Additional effects seen at 700 ppm included decreased body weight (males) and body weight gain (males and females); increased food consumption (when measured as g/kg/day); increased grooming, increased rearing, and decreased rotarod time in males; decreased motor activity in females.

Based on the effects seen in this study, the LOEL for systemic effects (increases in clinical signs) was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for neurotoxicity (FOB findings and decreased motor activity) was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for erythrocyte cholinesterase inhibition was 700 ppm (48.63 or 58.27 mg/kg/day for males or females, respectively), with a NOEL of 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively). The LOEL for plasma cholinesterase inhibition was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for brain cholinesterase inhibition was 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively), with the NOEL less than 5 ppm (the lowest dose tested).

The study is classified as acceptable for subchronic neurotoxicity in rats.

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