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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: MAY 26,1999

MEMORANDUM

SUBJECT: **ACEPHATE:** Review of 21-Day Dermal Toxicity in Rats--Main Study (MRID No. 44541101) and 5-Day Pilot Study/Short- and Intermediate-Term Dermal Risk Assessments

TO: Pauline Wagner, Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

Jess Rowland, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

Felicia Fort, Risk Assessor
Registration Action Branch 1
Health Effects Division (7509C)

and

Loan Phan, PM Team Reviewer
Special Review and Reregistration Division (7508W)

FROM: Nancy E. McCarroll Nancy E. McCarroll 5/26/99
Toxicology Branch 1
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D. Whang Phang 5/27/99
Branch Senior Scientist
Reregistration Action Branch 1
Health Effects Division (7509C)

May 26, 1999

Registrant: Valent U.S.A. Corp.
Chemical: Acephate
DP Barcode: D255979 PC Code: 103301

ACTION: Review the 21-Day Dermal Toxicity Study in Rats (main study); include an assessment of the 5-day pilot study.

SUMMARY: On December 11, 1997, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) selected the dermal NOAEL of 12 mg/kg/day for Acephate from the 21-day dermal toxicity study in rats for the Short-and Intermediate-Term dermal risk assessments based on brain cholinesterase inhibition at the next higher dose of 60 mg/kg/day (see HED document No. 012453). Since that meeting, the Registrant has requested that the Agency reconsider both the main 21-day study and the 5-day pilot study for the dermal exposure scenarios (see response to EPA's Draft Document Titled ACEPHATE.HED Risk Assessment and Disciplinary Chapters for the Reregistration Eligibility Decesion (RED) Document, dated March 8, 1999).

CONCLUSION: It was concluded by EPA reviewers and the expert dermal toxicologist member of the HIARC, Dr. P.V. Shah that the effects at 60 mg/kg/day were valid in the main 21-day dermal study because cholinesterase inhibition (ChEI) occurred in a dose-related manner, was significant and ChE activity was seen in the most sensitive parameter (brain). The systemic LOAEL was,therefore,set at 60 mg/kg/day;the NOAEL was 12 mg/kg/day. By contrast, the pattern of brain ChEI in the 5-day dermal pilot was not clearly evident because of the variability in the data at 50 and 150 mg/kg/day (i.e., standard deviations were in excess [≈2.5x] of the standard deviations for the other groups [0.64-0/79] and in all groups in the main study [0.55-0.83]. **The NOAEL and LOAEL (12 and 60 mg/kg/day, respectively) selected by the HIARC for the short-term and intermediate-term dermal exposure scenarios (see HED Document No. 012453) remain unchanged.**

Presented below is the Citation and Executive Summary for the reviewed study (MRID No. 44525301); the Data Evaluation Report is attached. The findings of this study are acceptable and satisfy the guideline requirement for a 21-day dermal study in the rat.

CITATION: Blaszcak, D. (1998) Acephate Technical: A 21-Day Dermal Toxicity Study in the Rat. Huntingdon Life Sciences. Project No. 97-2547, February 5, 1998. MRID 44541101. Unpublished.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 44541101], Acephate Technical [97.8% a.i.] was administered to 10 Sprague-Dawley rats/sex/dose via the skin [≈10% of the body surface area] at dose levels of 0, 12, 60, and 300 mg/kg/day for 21 days [6 hours per day, 5 days per week for 3 consecutive weeks]. Dose selection was based on the findings of a 5-day pilot study conducted with groups of three rats/sex/dose dermally exposed to 5, 50, 150 or 300 mg/kg/day. In this study, there were no effects on survival, clinical signs or body weight and there was no indication of dermal irritation at any dose. However, at the highest dose tested, females displayed a decrease in both plasma (83% of control) and brain (86% of control) cholinesterase while brain cholinisterase in the high-dose males was 90% of control.

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In the main 21-day study, all rats survived until study termination, and there were no clinical signs of toxicity. There was no dermal response. No adverse effects were observed on body weight, food consumption, hematology, clinical chemistry, and organ weights, and gross and microscopic findings were comparable among the groups for both sexes. At the high-dose level, both sexes displayed slight decreases in plasma and RBC cholinesterase values at study termination, although statistical significance was not attained. There was a decrease in brain cholinesterase activity in the mid- and high-dose groups of both sexes compared to the controls, and the decrease was significant (mid-and high dose females and high dose males) and dose-related. **The systemic NOAEL is 12 mg/kg/day, based on a slight decrease in brain cholinesterase activity at the systemic LOAEL of 60 mg/kg/day in females. The dermal toxicity NOAEL is 300 mg/kg/day, the highest dose tested.**

This 21-day dermal toxicity study is classified Acceptable, and it satisfies the guideline requirement for a repeated dose [21-day] dermal toxicity study [§82-2; OPPTS 870.3200] in rats.

ATTACHMENT: Data Evaluation Record, MRID No. 44541101

5/27/1999

[ACEPHATE] REPEATED DOSE [21-DAY] DERMAL TOXICITY [§82-2] OPPTS 870.3200

EPA Reviewer: Linda L. Taylor, Ph.D. 5/26/99
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. 5/27/99
Branch Senior Scientist, Reregistration Branch I, Health Effects Division (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose [21-Day] Dermal Toxicity Study OPPTS 870.3200 (rodent) [§82-2]
DP BARCODE: D255979 SUBMISSION CODE: S359396
P.C. CODE: 103301 TOX. CHEM. NO.: 002A
TEST MATERIAL (PURITY): [97.8%]
SYNONYMS: dimethyl acetylphosphoramidothioate CAS Registry No: 30560-19-1

CITATION: Blaszcak, D. (1998) Acephate Technical: A 21-Day Dermal Toxicity Study in the Rat. Huntingdon Life Sciences. Project No. 97-2547, February 5, 1998. MRID 44541101. Unpublished.

SPONSOR: Valent U.S.A. Corporation

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study [MRID 44541101], Acephate Technical [97.8% a.i.] was administered to 10 Sprague-Dawley rats/sex/dose via the skin [≈10% of the body surface area] at dose levels of 0, 12, 60, and 300 mg/kg/day for 21 days [6 hours per day, 5 days per week for 3 consecutive weeks]. Dose selection was based on the findings of a 5-day pilot study conducted with groups of three rats/sex/dose dermally exposed to 5, 50, 150 or 300 mg/kg/day. In this study, there were no effects on survival, clinical signs or body weight and there was no indication of dermal irritation at any dose. However, at the highest dose tested, females displayed a decrease in both plasma (83% of control) and brain (86% of control) cholinesterase while brain cholinisterase in the high-dose males was 90% of control.

In the main 21-day study, all rats survived until study termination, and there were no clinical signs of toxicity. There was no dermal response. No adverse effects were observed on body weight, food consumption, hematology, clinical chemistry, and organ weights, and gross and microscopic findings were comparable among the groups for both sexes. At the high-dose level, both sexes displayed slight decreases in plasma and RBC cholinesterase values at study termination, although statistical significance was not attained. There was a decrease in brain cholinesterase activity in the mid- and high-dose groups of both sexes compared to the controls, and the decrease was significant (mid-and high dose females and high dose males) and dose-related. **The systemic NOAEL is 12 mg/kg/day, based on a slight decrease in brain cholinesterase activity at the systemic LOAEL of 60 mg/kg/day in females. The dermal toxicity NOAEL is 300 mg/kg/day, the highest dose tested.**

This 21-day dermal toxicity study is classified Acceptable, and it satisfies the guideline requirement for a repeated dose [21-day] dermal toxicity study [§82-2; OPPTS 870.3200] in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Technical Acephate
Description: white powder
Lot/Batch #: R23044/VS-9B-40
Purity: 97.8% ai.
Stability of compound: expiration date May 8, 1998
Supplier: Valent U.S.A. Corporation
CAS #: 30560-19-1

2. Vehicle and/or positive control: 0.9% sodium chloride for Injection, USP
Description: clear liquid
Lot #: 51529
Purity: 0.9%
Stability of compound: expiration date October, 1997
Supplier: Sanofi Animal Health, Inc.

3. Test animals: Species: rat
Strain: Sprague-Dawley Crl:CD®BR VAF/Plus®
Age: 6 weeks old [main]/7 weeks old [pilot] at study initiation
Body weight at study start: males [main 188-231 g/ pilot 273-299 g]; females [main 126-165 g/pilot 173-208 g] at study initiation
Source: Charles River Laboratories, Kingston, NY
Housing: individual [during study]
Diet: PMI Certified Rodent Diet #5002 (meal) [PMI Feeds, Inc.] ad libitum, except prior to clinical pathology determinations
Water: tap, ad libitum
Environmental conditions: standard laboratory
Acclimation period: approximately two weeks

B. STUDY DESIGN

1. In life dates - start: July 9, 1997 [pilot]/ July 30, 1997 [main]; end: July 14, 1997 [pilot]/August 21, 1997 [main].

2. Animal assignment

Rats were assigned to the test groups [randomly] by evaluation of pretest cholinesterase values. The test groups consisted of one control group [0.9% sodium chloride] and 3 dose levels [12, 60, and 300 mg Acephate/kg body weight/day]. The dose volume for all groups was 1 mL/kg. The doses were adjusted, based on the most recent body-weight data. There were 10 rats/sex/group. The rats were dosed five days a week by dermal application for three weeks [15 or 16 doses, depending on day of sacrifice].

3. Dose preparation and analysis

Test material was mixed with the vehicle to yield concentrations of 12, 60, and 300 mg/mL. The preparations were prepared daily on the day of dosing. Control and treatment rats received the vehicle at a dosing volume of 1 mL/kg/day. The dose levels utilized in the study were 12 mg/kg/day, 60 mg/kg/day, and 300 mg/kg/day.

Homogeneity [prior to study initiation], stability [low- and high-dose solutions stored for 0, 20, 27, 34, and 41 days after preparation], and concentration [all dose levels from solutions prepared on days 1, 8, 15, and 22] analyses were performed.

The doses were selected on the basis of the results of a 5-day dermal exposure, range-finding, study in which 3 rats/sex/group were exposed dermally to the test material [dermally once a day for 5 consecutive days] at doses of 0 [saline], 5, 50, 150, and 300 mg/kg/day in a manner similar to that in the definitive study. Body weights were recorded pre-test [twice] and on day 6; mortality checks were performed twice a day, and observations for pharmacologic/toxicologic effects and dermal evaluations for irritation were performed daily. Cholinesterase determinations were performed on all rats at study termination; erythrocyte and brain cholinesterase determinations were calculated using dilution factors of 25 and 50, respectively. All rats were subjected to a complete necropsy at study termination.

Pilot Study Results: There were no deaths or clinical signs of toxicity. Body weights [Table 1] were comparable among the groups for both sexes, and there were no apparent effects on body-weight gain in either sex. Signs of dermal irritation were not reported for either sex, and there were no dose-related necropsy findings. At the high-dose level, females displayed a decrease in both plasma [83% of control] and brain [86% of control] cholinesterase [Table 2]. Males displayed a decrease in both plasma and brain cholinesterase at the three highest dose levels, but there was no dose response. From these results, it is not clear to this reviewer why the limit dose [1000 mg/kg/day] was not utilized in the definitive study.

Table 1. Body-Weight Data [range-finding study]

Dose [mg/kg/day]/Sex/Parameter	0	5	50	150	300
MALES					
body weight					
day -7	223.3±5.4	235.4±6.4	235.9±7.4	228.0±10.1	229.3±7.1
day 0	283.3±10.3	287.5±8.6	286.2±12.9	279.1±13.8	284.8±10.5
day 6	322.0±21.1	320.6±9.6	318.5±12.1	313.5±17.7	321.4±11.8
body-weight gain♪					
days -7-0	59.9±5.5	52.1±4.7	50.3±8.7 [84]	51.1±5.7 [85]	55.5±3.6 [93]♪
days 0-6	38.8±11.0	33.1±6.1	32.4±4.8 [84]	34.4±4.5 [89]	36.5±5.1 [94]
FEMALES					
body weight					
day -7	168.8±13.0	166.7±7.9	163.5±8.8	161.0±6.2	158.3±5.5 [94]
day 0	193.3±12.9	190.1±11.9	186.1±12.1	189.9±6.7	187.9±7.3
day 6	207.0±22.4	201.1±8.9	202.4±13.8	206.0±1.6	204.5±13.5
body-weight gain♪					
days -7-0	24.4±2.1	23.4±5.5	22.7±3.3	28.9±4.6	29.6±1.9
days 0-6	13.7±9.8	11.0±5.5	16.2±9.3	16.2±5.1	16.6±6.3

♪ [% of control]; calculated by reviewer using data from Appendix M [pages 344-345] of the report

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Table 2. 5-Day Dose Range-Finding Results [cholinesterase data; IU/mL]]					
Dose/Sex/Parameter	0 mg/kg/day	5 mg/kg/day	50 mg/kg/day	150 mg/kg/day	300 mg/kg/day
MALES					
pre-test					
PChE	0.628±0.148	0.649±0.172	0.622±0.133	0.604±0.118 [4]	0.635±0.078
RChE	1.296±0.174	1.354±0.508	1.799±0.526	1.384±0.112	1.354±0.150
termination					
PChE	0.542±0.135	0.603±0.165	0.502±0.085 [7]	0.475±0.085 [12]	0.518±0.052 [4]♪
RChE	1.167±0.056	1.188±0.184	1.159±0.083	1.096±0.190	1.188±0.033
BChE [IU/g]	18.444±1.035	18.894±0.655	**17.122±1.304 [7]**	**16.339±0.150 [11]**	**16.639±0.786 [10]**
FEMALES					
pre-test					
PChE	1.053±0.092	1.064±0.243	1.124±0.193	1.034±0.208	1.048±0.284
RChE	1.325±0.120	1.329±0.367	1.334±0.007	1.371±0.297	1.271±0.165 [4]
termination					
PChE	1.364±0.134	1.414±0.448	1.203±0.407	1.299±0.276	1.128±0.399 [17]
RChE	1.104±0.069	1.204±0.200	1.075±0.076	1.334±0.350	1.038±0.076 [6]
BChE [IU/g]	17.011±0.648	18.717±0.788	17.500±2.447	19.178±2.459	**14.711±0.642 [14]**

♪ [% inhibition]; data from Appendix M pages 346-347 of the report

4. Dose administration

Prior to randomization [test day 0] and approximately twice weekly thereafter, the dorsal skin of each rat was clipped free of hair. The clipped area was stated to be greater than 10% of the body surface. Doses of test material or vehicle were applied directly to the clipped skin and spread evenly over the treatment area of each rat. Following application, the test site was covered with gauze, which was secured with Elastoplast® tape. After a minimum of 6 hours exposure, the gauze, tape, and residual test/control material were removed.

5. Statistics - Body weight, body-weight change, food consumption, clinical laboratory values, cholinesterase determinations, and organ weight: equality of means made by appropriate one-way analysis of variance technique, followed by a multiple comparison procedure; if needed. First, Bartlett's test was performed to determine if groups had equal variance. If equal, parametric procedures [standard one-way ANOVA suing the F distribution to assess significance; if significant differences were indicated, Dunnett's test was used to determine which means were significantly different from control] were used; if not, nonparametric procedures [Kruskal-Wallis test was used, and if differences were indicated, Dunn's summed rank test was used to determine which treatments differed from control] were used. A statistical test for trend in dose levels was performed also. In the parametric case, standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's test for monotonic trend was used. Exceptions: Statistical evaluations were not performed when the standard deviation for the control was 0. Dose groups were eliminated from statistical analysis if their standard deviation was 0 and/or the number of animals was ≤2.

C. METHODS

1. Clinical observations: The rats were observed twice daily for general health/mortality, and signs of severe toxic/pharmacologic effects. Physical examinations, which consisted of removal from cage and unusual behavior and physical signs were recorded for each rat, were performed twice pretest and weekly thereafter.

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2. Dermal observations: The application site of each rat was examined for signs of dermal irritation daily prior to dosing, and dermal irritation was evaluated and scored on test days 0, 1, 2, 3, 6, 7, 14, 21, and 22 or 23. The test site was examined with respect to lesions [erythema, edema] and other dermal findings [atonia, desquamation, fissuring, eschar exfoliation, tissue damage].

3. Ophthalmoscopic examination: Lids, lacrimal apparatus, and conjunctive were examined grossly. The cornea, lens, iris, anterior chamber, vitreous humor, retina, and optic disc were examined by indirect ophthalmoscopy.

4. Body weight: Each rat was weighed twice pretest, weekly during dosing, and just prior to necropsy [after fasting].

5. Food consumption: Individual food consumption was recorded weekly, beginning one week prior to treatment.

6. Clinical pathology evaluations: Prior to necropsy [days 22/23] and following an overnight fast, blood was collected from each rat [via the orbital plexus (retrobulbar venous plexus) while under light CO_2/O_2 anesthesia] for hematology and serum chemistry analysis. Blood for coagulation parameter sampling was obtained *via* the abdominal aorta at necropsy under light CO_2/O_2 anesthesia from all rats. The CHECKED (X) parameters were examined. Absolute lymphocytes [total WBC x % lymphocyte value ÷ 100] and absolute segmented neutrophils [total WBC x % segmented neutrophil ÷ 100] were calculated.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Red cell morphology
	(Thromboplastin time)		Platelet estimate
X	(Activated partial thromboplastin time)		
X	(Prothrombin time)		

Blood Chemistry

	Electrolytes:		Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
X	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulin
X	Sodium	X	Glucose
	Iron		Phospholipids
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total Protein (TP)
X	Cholinesterase (ChE)	X	Triglycerides
	Creatine kinase (CK)	X	A/G ratio
X	Sorbitol dehydrogenase		Triiodothyronine [T3]
X	Alanine aminotransferase		Thyroxine [T4]
X	Aspartate aminotransferase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate lactate dehydrogenase (GLDH)		
	Ornithine carbamyltransferase (OCT)		
	Electrophoretic protein fractions		

Cholinesterase values [RBC and plasma] were determine for all rats on pretest day 6 and at study termination. Blood was obtained *via* the orbital sinus [retrobulbar venous plexus] under light CO_2O_2 anesthesia from each rat following an overnight fast. Brain cholinesterase values were determined for all rats at study termination. The right half of the brain was homogenized, and three samples of homogenate were collected for each rat. ChE parameters [brain, RBC, plasma] were measured using the Modified Ellman Method - Kinetic].

7. Sacrifice and Pathology

At study termination, all rats were sacrificed, following an overnight fast, *via* CO_2 inhalation followed by exsanguination. Each was subjected to a gross pathological examination, which included examination of the external surfaces of the body and all orifices; external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal, and pelvic cavities and neck; and the remainder of the carcass for the presence of macroscopic morphological abnormalities. The adrenals, brain, kidneys, liver, lungs, spleen, thymus, ovaries, and testes were weighed [all rats]. The CHECKED (X) organs and tissues from each rat were preserved for possible future histopathological examination, and the CHECKED (X) organs and tissues were examined microscopically for the control and high-dose rats. NOTE: On page 30 under 2.19.4 Tissues Preserved and Examined Histopathologically, the second sentence indicates that the slides of the indicated tissues were examined microscopically for **all** animals, but Table I shows that only the control [I] and high-dose [IV] groups were examined.

Digestive system		Cardiovasc./Hemat.		Neurologic	
X	Tongue	X	Aorta	X	Brain♦♦
X	Salivary glands✹	X	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow	X	Spinal cord♣
X	Stomach	X	Lymph nodes♦	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes/optic nerve
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		X	Adrenal gland
X	Cecum	X	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	Other	
X	Pancreas	X	Seminal vesicle	X	Bone/sternum/femur w/ joint
Respiratory		X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin [treated/untreated]
X	Lung	X	Vagina	X	Zymbal's gland
X	Nasal turbinates		Cervix	X	All gross lesions
X	Pharynx			X	Harderian gland
X	Larynx				Internal ear structures

♦ *mesenteric*; ♣ *cervical, thoracic, lumbar*; ✹ *submandibular*
♦♦ cerebrum, medulla/pons, cerebellum, optic chiasma

II. RESULTS

A. Analytical results: The mixing procedure was found to be adequate, and the dosing solutions were stable for at least 42 days at -20°C [dosing solutions were used on the day they were prepared]. The concentrations of the dosing solutions were found to be 103%-112% of the nominal concentration.

B. Observations: All rats survived until study termination. There were no clinical findings that could be attributed to treatment. Dermal irritation was not observed at any dose level in either sex. Ophthalmoscopic examinations performed pre-test showed all groups/both sexes to be within normal limits. At 3 weeks, ocular abnormalities were observed only in treated rats [focal retinopathy in one mid-dose male, one low-dose female, 3 mid-, and 3 high-dose females; conjunctivitis in one mid-dose male and two mid-dose females].

C. Body weight and weight gain: Comparable body weights and body-weight gains were observed among the groups for both sexes throughout the study [Table 3].

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Table 3. Body-Weight Data [grams]				
Dose/Sex/Parameter	0 mg/kg/day	12 mg/kg/day	60 mg/kg/day	300 mg/kg/day
MALES				
body weight				
week -1	151.2±7.4	152.1±11.3	154.6±6.7	153.7±6.2
week 0	206.5±12.3	208.5±5.9	210.0±12.1	206.8±9.1
week 1	260.9±13.4	266.4±13.0	266.3±12.6	263.5±13.6
week 2	305.9±18.9	312.7±20.9	314.4±16.8	311.4±17.0
week 3	345.7±22.7	351.5±28.8	354.1±21.1	346.2±20.0
body-weight gain				
weeks -1-0✱	55.3	56.4	55.4	53.1
weeks 0-1	54.4±4.2	57.9±10.3	56.3±3.6	56.7±7.9
weeks 0-3	139.2±12.6	143.0±26.2	144.1±14.4	139.4±15.4
FEMALES				
body weight				
week -1	108.5±8.6	108.3±8.8	107.8±7.3	107.4±11.1
week 0	147.8±12.0	146.2±9.5	146.2±6.7	146.6±10.8
week 1	173.6±13.8	170.1±9.6	169.0±7.5	171.8±13.8
week 2	194.6±14.3	193.8±10.8	190.5±12.3	194.1±14.1
week 3	209.3±15.6	210.7±12.6	213.6±16.1	208.8±14.7
body-weight gain				
weeks -1-0✱	39.3	37.9	38.4	39.2
weeks 0-1	25.8±5.8	23.9±3.4	22.8±4.9	25.2±5.5
weeks 0-3	61.5±7.5	64.5±8.4	67.4±13.5	62.2±9.6

✱ calculated by reviewer using mean data from Table 5 [Mean Body Weight Values] of the report [pages 58-59];
♪ [% of control]; data excerpted from Tables 5 & 6 [pages 58-61] of the report

D. Food consumption: There was no adverse effect of treatment on food consumption in either sex, but females ate more food on a gram/kg/day basis than the males.

E. Dermal observations: Dermal irritation was not observed.

F. Blood work

1. Hematology - Values for the various parameters were comparable among the groups for both sexes.

2. Clinical Chemistry - There were no apparent treatment-related differences observed in either sex.

G. Urinalysis: Not performed.

H. Cholinesterase: At the high-dose level in both sexes, there was a slight inhibition in plasma [males 5%; females 6%] and RBC [males 9%; females 13%] cholinesterase, but statistical significance was not attained. Brain cholinesterase values were reduced in both sexes at the mid- [males 5%; females 6%] and high-dose [males 9%; females 14%] levels, although statistical significance was attained only in the females at the mid-dose level but in both sexes at the high-dose level.

Table 4. Cholinesterase Data [IU/mL]				
Dose/Sex/Parameter	0 mg/kg/day	12 mg/kg/day	60 mg/kg/day	300 mg/kg/day
MALES				
pre-test				
PChE	0.622±0.125	0.664±0.097	0.695±0.105	0.667±0.125
RChE	1.525±0.295	1.519±0.304	1.499±0.251	1.514±0.250
termination				
PChE	0.609±0.150	0.622±0.136	0.610±0.101	0.578±0.085 [5]
RChE	1.308±0.451	1.267±0.349	1.304±0.415	1.185±0.167 [9]
BChE [IU/g]	18.270±0.806	18.173±1.183	**17.303±1.425 [5]♪**	**16.628±0.515** [9]**
FEMALES				
pre-test				
PChE	0.670±0.112	0.766±0.135	0.719±0.143	0.723±0.126
RChE	1.584±0.167	1.585±0.216	1.625±0.233	1.588±0.193
termination				
PChE	1.374±0.380	1.465±0.294	1.462±0.483	1.292±0.282 [6]
RChE	1.303±0.319	1.487±0.776	1.253±0.223 [4]	1.137±0.259 [13]
BChE [IU/g]	18.317±0.715	18.652±0.834	**17.137±0.722** [6]**	**15.787±0.553** [14]**

♪ [% inhibition]; ** p<0.01; data from Table 11, pages 78-81 of the report

I. Sacrifice and Pathology

1. Organ weight - Organ weights were comparable among the groups for both sexes [Table 5].

Table 5. Organ-Weight Data [grams]				
Sex/Dose/Organ	0 mg/kg/day	12 mg/kg/day	60 mg/kg/day	300 mg/kg/day
MALES				
brain	1.984±0.096	1.956±0.113	1.996±0.091	2.012±0.094
spleen	0.665±0.096	0.673±0.150	0.651±0.104	0.669±0.049
thymus	0.672±0.118	0.634±0.168	0.633±0.105	0.633±0.125
lungs	2.081±0.309	2.107±0.394	2.308±0.381	2.276±0.300
kidneys	2.782±0.243	2.964±0.278	2.877±0.294	2.720±0.493
liver	9.927±0.762	10.022±1.228	10.094±0.804	9.778±0.773
adrenals	0.0694±0.0081	0.0654±0.0121	0.0668±0.0121	0.0667±0.0091
testes	2.977±0.182	3.054±0.087	3.045±0.166	2.982±0.174
terminal body weight	311±21	318±27	318±19	313±19
FEMALES				
brain	1.819±0.052	1.771±0.075	1.785±0.099	1.803±0.087
spleen	0.472±0.079	0.422±0.054	0.436±0.072	0.494±0.099
thymus	0.498±0.077	0.496±0.109	0.525±0.154	0.495±0.074
lungs	1.741±0.218	1.682±0.242	1.703±0.300	1.742±0.250
kidneys	1.870±0.157	1.721±0.284	1.920±0.123	1.905±0.178
liver	6.693±0.409	6.455±0.379	6.572±0.732	6.824±0.533
adrenals	0.0685±0.0096	0.0746±0.0094	0.0719±0.0080	0.0781±0.0118
ovaries	0.0946±0.0176	0.0882±0.0262	0.0866±0.0179	0.0907±0.0158
terminal body weight	190±15	188±10	188±13	186±13

Data from Table 12 [pages 83-90] of the report

2. Gross pathology - There were no differences noted among the groups.

3. Microscopic pathology - No microscopic findings were reported in the skin. There was no apparent treatment-related pathology in either sex.

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III. DISCUSSION

A. Besides a decrease in cholinesterase activity in plasma, RBC, and brain, no other significant adverse effects were observed following exposure of rats to Acephate at dose levels up to 300 mg/kg/day via the skin for 21 days [6 hours per day, 5 days per week for 3 consecutive weeks]. There was no dermal response. No adverse effects were observed on body weight, food consumption, hematology, clinical chemistry, and organ weights, and gross and microscopic findings were comparable among the groups for both sexes. At the highest dose tested [300 mg/kg/day], both sexes displayed slight decreases in plasma and RBC cholinesterase values at study termination, although statistical significance was not attained and the magnitude of the decreases was small. There was a dose-related, statistically-significant [$p<0.01$] decrease in brain cholinesterase activity in both sexes compared to the control values in the high-dose groups, and a statistically-significant[$p<0.01$] decrease in brain cholinesterase activity in females at the mid-dose level [60 mg/kg/day] also.

With respect to the inhibition observed in brain cholinesterase in females at the mid-dose level, although the magnitude is small [6%], the number of females with a value less than 17.4 IU/g [the **lowest** value observed in the control group] was 5/10 at the mid-dose and 10/10 at the high-dose level. All of the low-dose females displayed a brain cholinesterase value greater than 17.4 IU/g. Additionally, the highest value observed in the 60 mg/kg/day females was less than 7 of the 10 control values and 8 of the 10 low-dose values [Table 6].

In order to determine whether the slight decrease in brain cholinesterase observed at the mid-dose level in females should be considered treatment-related, the results of the pilot study were evaluated more closely. In the pilot study, the brain cholinesterase values of the female control group are low relative to the values in the next three dose groups [Table 6] in the pilot study and the values of 18 of the 20 rats in the control and 12 mg/kg/day groups in the definitive study. Additionally, although the females in the 50 and 150 mg/kg/day dose groups in the pilot study displayed brain cholinesterase values that were greater than control, the standard deviations for these two groups [≈2.5] were in excess of the standard deviations observed for the other groups [0.64-0.79] in the pilot study and in all groups [0.55-0.83] in the definitive study. Because of the variability noted in the pilot study with respect to brain cholinesterase, the pilot study does not support a determination that the 60 mg/kg/day dose level is a no-effect level.

Table 6. Brain Cholinesterase Values [IU/g; females]

Individual Values in Pilot Study [**control values bolded**]	Individual Values in Definitive Study		
	Control	12 mg/kg/day	60 mg/kg/day
22.017	17.400	17.450	15.850
20.083	17.567	17.633	15.867
19.617	17.667	18.083	16.950
18.383	18.050	18.567	17.133
18.150	18.167	18.700	17.317
17.800	18.317	18.733	17.533
17.717	18.333	18.833	17.583
17.450	18.850	18.850	17.650
17.317	19.333	19.300	17.717
17.200	19.483	20.367	17.767
16.267			
15.217			

Data from Appendices I and M [pages 174, 175, 347] of the report

The systemic NOAEL is 12 mg/kg/day, based on a slight decrease in brain cholinesterase activity at the systemic LOAEL of 60 mg/kg/day. The dermal toxicity NOAEL is 300 mg/kg/day, the highest dose tested.

B. Study deficiencies - None that would adversely affect study interpretation. 013396