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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: July 27, 2000

MEMORANDUM

SUBJECT: **ACEPHATE:** Revised Review of 21-Day Dermal Toxicity in Rats (MRID No. 45134301) and Revised Review of the Subchronic Inhalation (Nose Only)Toxicity Study in the Rat (MRID No. 45134302)

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

and

Monica Alvarez, PM Team Reviewer
Special Review and Reregistration Division (7508W)

FROM: Nancy E. McCarroll Nancy E. McCarroll 7/28/00
Toxicology Branch 1
Health Effects Division (7509C)

THRU: Alberto Protzel, Ph.D. 7/28/00
Branch Senior Scientist
Toxicology Branch 1
Health Effects Division (7509C)

Registrant: Valent U.S.A. Corp. Submission No. S580552
Chemical: Acephate
DP Barcode: D267731 PC Code: 103301

ACTION: Revise the 21-day dermal toxicity study in rats (MRID No. 45134301) data evaluation record (DER) and the 28-day subchronic inhalation (nose only) toxicity study in the rat (MRID No. 45134302) DER.

July 28, 2000

CONCLUSION: Attached are the electronic copies of the **revised** DERs on the above studies. These studies have been revised as per recommendations made at the July 19, 2000 Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) meeting for Acephate. It should be noted that these revisions neither altered the integrity nor outcome of the 21-day dermal toxicity study in rats (MRID No. 45134301) or the 28-day subchronic inhalation (nose only) toxicity study in the rat (MRID No. 45134302).

EPA Reviewer: Linnea J. Hansen, Ph.D.
Toxicology Branch I (7509C)
EPA Secondary Reviewer: P.V. Shah, Ph.D.
Registration Action Branch I (7509C)

Nancy E. McCall & L. Hansen, Date 7/28/00
for P.V. Shah, Date 7/28/00

014258

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose (21-Day) Dermal Toxicity. OPPTS 870.3200 [§82-2]

DP BARCODE: D266531
P.C. CODE: 103301

SUBMISSION CODE: S580552
TOX. CHEM. NO.: 002A

TEST MATERIAL (PURITY): Acephate, tech.(98.8% a.i.)

SYNONYMS: O, S-dimethyl acetylphosphoroamidothioate; Ortho 12420, Orthene® and many other trade names

CITATION: Hoffman, Gary (2000) Acephate Technical: A 21-Day Dermal Toxicity Study in Rats. Huntingdon Life Sciences, East Millstone, NJ. Laboratory Project Identification No. 99-2637. May 26, 2000. MRID 45134301. Unpublished.

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

EXECUTIVE SUMMARY: In a repeated dose dermal toxicity study (MRID 45134301), acephate (tech., 98.8% a.i.) was applied to the shaved dorsal skin of 10 Crl:CD®(SD)IGS BR rats/sex/dose in 0.9% saline vehicle (1 ml/kg body weight) at concentrations of 0, 20, 30, 40 or 50 mg/kg/day, 5 days/week, 6 hrs/day over 21 days (total of 16 applications).

No treatment-related mortality or clinical signs (including local dermal irritation at the application site) and no effects on body weights, food consumption, cholinesterase activities (plasma, RBC or brain) or gross observations at necropsy were reported. Hematology, clinical chemistry, urinalysis, organ weight determinations and microscopic examination of tissues were not performed. **The systemic toxicity (ChE) LOAEL is >50 mg/kg/day (HDT) and the NOAEL is 50 mg/kg/day. The LOAEL for local dermal irritation is >50 mg/kg/day and the NOAEL is 50 mg/kg/day.**

This 21-day dermal toxicity study is classified **Acceptable/nonguideline**. Although by itself it does not satisfy the guideline requirement for a 21-day dermal toxicity study (§82-2) due to lack of evaluation of blood, organ weights and microscopic pathology, the study is acceptable when considered together with an earlier 21-day dermal toxicity in the rat (MRID 44541101; see review in HED Doc. No. 013396). **The systemic toxicity (ChE) NOAEL of 50 mg/kg/day for dermal exposure identified in this study may therefore be used for regulatory purposes in *lieu* of the previously determined NOAEL of 12 mg/kg/day from the earlier study which was based on slight but statistically significant inhibition in brain cholinesterase activity in**

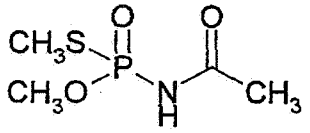
females.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. A Flagging statement was not provided. This does not affect the integrity of the study. There were no adverse effects observed in this study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Acephate
   Description: technical, white solid.
   Lot/Batch #: VJI-001TG-21 (Valent; Source No. 71047)
   Purity: 98.8% ai.
   Stability of compound: The expiration date for the test material provided in this study was September 9, 2000. Test material (dry) was stored at room temperature. Stability data was reportedly on file with the sponsor and not provided in the report. However, the test material was shown to be stable for the duration of this study under the storage conditions used (see dosing solution analysis).
   CAS #: 30560-19-1
   Structure:



2. Vehicle: 0.9% saline. Lot/Batch #G949941 (VWR Scientific)

3. Test animals: Species: rat
   Strain: Crl:CD®(SD) IGS BR (Outbred VAF/Plus® Sprague-Dawley -derived (CD®)
   Age and weight at study initiation: approximately 7 weeks. Males range 223.3-255.0 g; females 159.1- 200.7 g
   Source: Charles River Laboratories, Kingston, NY
   Housing: individually in suspended stainless steel wire mesh cages
   Diet: PMI Nutrition International Certified Rodent Diet #5002 *ad libitum*
   Water: tap water *ad libitum*
   Environmental conditions: Temperature: 21 to 24°C
   Humidity: 38% to 66%
   Air changes: not indicated
   Photoperiod: 12 hr light/12 hr dark
   Acclimation period: 9 or 10 days

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B. STUDY DESIGN:

1. In life dates - start: January 5 (males) and 6 (females), 2000; end: January 27 (males) and 28 (females), 2000

2. Animal assignment

Animals were assigned to the test groups in Table 1 by a computerized randomization program that ranked body weights to equalize mean body weights.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Dosing solution (mg/mL)	Dose to Animal (mg/kg/day)	Male	Female
Control	0	0	10	10
Low	20	20	10	10
Mid 1	30	30	10	10
Mid 2	40	40	10	10
High	50	50	10	10

Animals were shaved about 24 hrs prior to the initiation of dosing on a 5 x 5 cm area of the dorsal skin between the scapula and the hipbone and half-way down the flank of each side (approximately 10% of the body surface area). As needed (in general, about twice weekly), animals were shaved again during the study, each time after the 6 hr dosing period. Test material was applied to the shaved area in a constant dose volume of 1 mL/kg body weight using the appropriate solution for each dose level. Controls received physiological saline only at the same volume as the dosed animals. Dosing volume was determined based on the most recent body weight for each animal. The treated skin was protected during exposure with 8-ply gauze held with Elastoplast®. Bandages were removed and the treated areas of skin were wiped with dry gauze at the end of each 6 hr exposure period.

The study protocol deviation section noted that due to inclement weather on January 25, (1) animals were exposed for 4 hrs instead of 6 and (2) blood and brain cholinesterase collection and termination for males and females were delayed one day, giving 16 exposures instead of 15 as specified in the protocol.

3. Rationale for dose selection

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The doses selected for this study were based on a previously conducted 21-day dermal toxicity study (HLS Study No. 97-2547; MRID 44541101; reviewed in HED Doc. No. 013396), in which a NOAEL of 12 mg/kg/day was identified based on cholinesterase inhibition in the brain at the LOAEL of 60 mg/kg/day. No dermal irritation was observed at any dose tested (HDT = 300 mg/kg/day). Doses intermediate to these two values (20, 30, 40 and 50 mg/kg/day) were chosen for this new study to refine the NOAEL from the earlier study.

4. Dosing solution preparation and analysis

Dosing solutions for each dose group were prepared once at the study start by dissolving appropriate amounts of test substance in 0.9% saline vehicle to achieve desired concentrations. Daily aliquots were stored frozen and thawed on the day of dosing just prior to administration. Homogeneity of low and high dose preparations used in the study was tested at the beginning of the study. Triplicate samples from the top, middle and bottom of the 20 and 50 mg/kg/day dosing solutions (total of 9 samples/batch) were analyzed. Duplicate samples from a preliminary set of high and low dose solutions were also tested. Concentrations of the dosing solutions for all dose groups including controls were analyzed in duplicate samples just prior to study initiation and at study termination. Stability of the test material in the dosing solution was determined by comparing the initial analytical concentration with the analytical concentrations of the solutions that had been stored frozen for 23 days.

Results - Homogeneity Analysis: The dosing solutions tested (20 and 50 mg/kg/day groups) were shown to be homogeneous. All of the samples tested were within <7% of the nominal concentration; most varied by only a few percent. The mean values for the top, middle and bottom of each dosing solution were 97.3%, 96.1% and 96.7% (20 mg/kg/day dose solution) and 96.1%, 95.7% and 97.5% (50 mg/kg/day solution). Individual analytical values for the study dosing solutions ranged from 93.5% to 99.2% of the target concentration (duplicate samples from the preliminary solutions ranged from 97.7% to 103.8% of nominal).

Stability Analysis: The test material was shown to be stable in the 0.9% saline vehicle under the storage conditions of this study. At study termination, the concentrations of the dosing solutions were 108.6%, 108.9%, 109.4% and 106.3% of the target concentrations for the 20, 30, 40 and 50 mg/kg/day dose groups, respectively (see Concentration Analysis, below, for concentrations at the study start).

Concentration Analysis: The concentrations of the dosing solutions used in this study were all within acceptable variability from the target concentrations. The mean concentrations of the dosing solutions used in this study (measured at the study start) were 101.0%, 107.9%, 101.7% and 107.7% of the target concentrations for the 20, 30, 40 and 50 mg/kg/day dose groups, respectively. The individual samples tested ranged

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between 97.7% and 108.9%.

The analytical data therefore indicate that the mixing procedure was adequate and that the variance between target and actual dosage to the animals was acceptable.

4. Statistics - Mean body weight, mean food consumption and mean blood and brain cholinesterase values were analyzed statistically. Data were analyzed using Bartlett's test to evaluate equality of variance. When variances were equal, a standard one-way ANOVA using the F ratio to assess significance was performed. If significant differences among the means were identified, this was followed by Dunnett's test or Williams test to evaluate significant differences from controls. When variances were unequal, Kruskal-Wallis test was performed. If significant differences among the means were identified, it was followed by Shirley's test, Dunn's test or the Pairwise Comparison with Boneferroni Correction to evaluate significant differences from controls. Statistical significance was identified at the 1% significance level for Bartlett's test and at the 5% and 1% level for all other tests.

C. METHODS:

1. Observations:

Animals were inspected twice daily for mortality and general condition and once daily for clinical cage-side signs of toxic or pharmacologic effects. A more detailed examination was conducted weekly. Treated skin was examined for signs of irritation daily for the first week of treatment, once weekly thereafter (prior to daily dosing) and just prior to necropsy.

2. Body weight

Animals were weighed twice prior to study start, weekly during treatment and at termination on study day 23.

3. Food consumption

Food consumption for each animal was determined weekly based on a 6-day interval for each week, beginning during the week prior to treatment. Mean daily diet consumption was calculated both as g food/kg body weight/day and as g/animal/day. Food efficiency was not determined.

4. Ophthalmoscopic examination

An ophthalmoscopic examination was not performed.

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5. Hematology and clinical chemistries

Analysis of hematology and blood chemistries was not performed in this study.

6. Cholinesterase evaluations

Plasma, RBC and brain cholinesterase activities were evaluated at study termination on study day 23 (one day after the last exposure). All samples were assayed using the Ellman procedure. Samples were collected and processed as follows:

a. Blood - after an overnight fast, blood was collected from the orbital sinus of each animal under light CO_2 anesthesia. Samples were heparinized, separated into plasma and cellular components by centrifugation and frozen until analysis.

B. Brain - at study termination, the right half of the brain was homogenized and stored frozen until analysis. Left brain and a portion of the homogenate were retained for up to 6 months for possible future analysis, if necessary.

7. Urinalysis

Urinalysis was not performed in this study.

8. Sacrifice and Pathology

All animals were subjected to gross pathological examination at termination on study day 23. No histological examination or organ weight measurements were conducted.

II. RESULTS

A. Observations:

1. Toxicity - No treatment-related clinical signs of toxicity were reported. Treatment-related dermal irritation was not observed: only 1 female at 40 mg/kg/day had very slight erythema and desquamation during study week 2 but no animals showed irritation prior to this, nor at study termination.

2. Mortality - No mortality was observed during this study.

B. Body weight and weight gain:

There were no treatment-related changes in mean body weights or in weight gain at any

dose tested. At day 20, mean body weights (control to high dose, respectively) were 352, 349, 345, 347 and 355 g, males and 233, 232, 233, 231 and 233 g (females).

C. Food consumption:

There were no treatment-related alterations in weekly food consumption in g/animal/day or in g/kg body weight/day. A statistically significant increase in g/kg/day for females on day 6 was considered sporadic since it was only observed at one time point and was of a small magnitude (6% above controls).

D. Cholinesterase evaluations

No treatment-related changes in plasma, erythrocyte or brain cholinesterase activities were reported at any dose tested. At 30 mg/kg/day in females, mean erythrocyte activity was decreased relative to controls (-21.2%) and plasma activity was increased (+10.9%), and plasma and erythrocyte activities were decreased in females by about -11% at 40 mg/kg/day, but these were considered sporadic due to the lack of dose-response (none were statistically significant).

E. Macroscopic pathology

No treatment-related gross pathology findings were observed.

III. DISCUSSION

A. The purpose of this study was to refine the systemic toxicity (ChE) NOAEL identified in a previously conducted 21-day dermal toxicity study in rats (MRID 44541101; reviewed in HED Doc. No. 013396), in which a NOAEL of 12 mg/kg/day was identified based on slight but statistically significant inhibition of brain cholinesterase activity in females at the LOAEL of 60 mg/kg/day. Both males and females showed inhibition of plasma, RBC and brain cholinesterase activities at 300 mg/kg/day (HDT). The current study assessed the most sensitive endpoint, cholinesterase inhibition in brain and blood, at doses intermediate to 12 and 60 mg/kg/day (20, 30, 40 and 50 mg/kg/day). Only clinical signs of toxicity (including local dermal irritation of the treated skin), mortality, body weight, food consumption, cholinesterase (blood and brain) and gross observations at necropsy were evaluated.

TB-I agreed with the conclusions of the study author that there were no treatment-related effects on clinical signs of toxicity (including local dermal irritation at the application site), mortality, body weight, food consumption, brain and blood cholinesterase activities or gross pathology at the doses tested (up to 50 mg/kg/day). The study is classified **Acceptable/nonguideline.** Although this was not a guideline study due to the limited parameters evaluated, it is considered acceptable for establishing a systemic toxicity

(ChE) NOAEL for dermal exposure for risk assessment purposes, when considered together with the previous 21-day dermal toxicity study. Based on the results of the previous study, effects other than inhibition of blood or brain cholinesterase activities are not anticipated to occur at dose levels that cause cholinesterase inhibition. **The systemic toxicity (ChE) NOAEL of 50 mg/kg/day (HDT) for repeated dermal exposure (§82-2) therefore replaces the systemic toxicity (ChE) NOAEL of 12 mg/kg/day from the previous dermal toxicity study on acephate.** The LOAEL of 60 mg/kg/day in the previous study is probably a threshold effect level, based on small but statistically significant decrease in brain cholinesterase activity in females only and lack of inhibition in the new study at 50 mg/kg/day. The LOAEL for local dermal irritation in this study was >50 mg/kg/day and NOAEL was 50 mg/kg/day, consistent with the dermal irritation NOAEL of 300 mg/kg/day (HDT) in the previous study.

B. Study deficiencies: Several of the standard parameters included in the guideline §82-2 repeated dose dermal toxicity study protocol were not performed in this study, including hematology, clinical chemistries, organ weights and microscopic pathological evaluation and it is therefore not a guideline study. However, the study was otherwise well-conducted and when taken together with the previously conducted 21-day dermal toxicity study, MRID 44541101, is considered acceptable for regulatory purposes. The NOAEL identified in this study therefore may be used as the systemic toxicity (ChE) NOAEL for a repeated-dose dermal toxicity study because inhibition of brain cholinesterase activity, the most sensitive endpoint identified in the previous study (NOAEL 12 mg/kg/day and LOAEL 60 mg/kg/day), was evaluated in this study.

Protocol deviations identified in this study report (due to inclement weather on January 25, 2000, dosing animals for 4 hrs instead of 6 hrs that day; and delaying of terminal sacrifice and assessment of cholinesterase for one day until Jan. 26, resulting in 16 instead of 15 exposures) do not alter the conclusions of this study.

014258

[Acephate, tech.] Subchronic Inhalation Study [82-4(a)]

EPA Reviewer: Linnea J. Hansen
Toxicology Branch I (7509C)
EPA Secondary Reviewer: John E. Whalan
Registration Action Branch II (7509C)

Mary L. McCarroll for L. Hansen, Date 7/25/00
Date 7/28/00

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - rat; OPPTS 870.3465 (rodent) [§82-4(a)]

DP BARCODE: D266532
P.C. CODE: 103301

SUBMISSION CODE: S580554
TOX. CHEM. NO.: 002A

TEST MATERIAL (PURITY): Acephate, tech. (98.8% a.i.)

SYNONYMS: O, S-dimethyl acetylphosphoroamidothioate; Ortho 12420, Orthene® and many other trade names

CITATION: Hoffman, Gary (2000) A 4-Week Nose-Only Inhalation Toxicity Study in Rats with Acephate Technical. Huntingdon Life Sciences, East Millstone, NJ. Laboratory Project Identification No. 99-6124, May 26, 2000. MRID 45134302. Unpublished.

SPONSOR: Valent Corporation, Walnut Creek, CA

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 45134302) acephate (tech., 98.8% a.i.) aerosol was administered by nose-only inhalation exposure to 10 Crl:CD®(SD)IGS BR rats/sex/concentration at levels of 0.000, 0.001064, 0.003123 or 0.005550 mg/L (target concentrations of 0.000, 0.001, 0.003 or 0.005 mg/L) for 4 weeks (5 days/week and 6 hrs/day; total of 20 exposures).

At 0.003123 mg/L, slightly decreased brain cholinesterase activity in males (-9.9% less than controls, $p<0.01$; females showed a very slight but not significant decrease of -5.2%); plasma cholinesterase in males on days 1 and 5 (-13.5% and -17.1%) and erythrocyte activity in females on day 5 (-21.4%; $p<0.05$) were observed. At 0.005550 mg/L, inhibition of cholinesterase activity in plasma (males -13.5%, $p<0.05$ to -18%, $p<0.01$ on days 1 and 5), erythrocytes (females -30%, day 5) and brain (-14.3%, males and -13.1%, $p<0.01$) was observed along with labored breathing in 25% to 33% of the animals during exposure on 3 days during the last week of the study. (A decrease of -11.6%, $p<0.05$, in plasma cholinesterase activity in males on day 5 at 0.001064 mg/L was considered insufficient for establishing an adverse effect). There were no treatment-related effects on body weight/weight gain, food consumption, organ weights, gross pathology or microscopic findings in the selected tissues that were examined (see DER). Ophthalmological examinations, hematology, clinical chemistry and a complete histopathology examination were not performed. **The ChE LOAEL is 0.003123 mg/L, based on inhibition of plasma and brain cholinesterase activities in males and erythrocyte cholinesterase in**

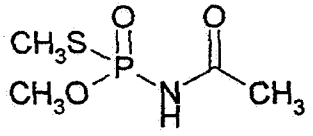
females. The ChE NOAEL is 0.001064 mg/L.
This subchronic inhalation toxicity study in the rat is classified **Acceptable/nonguideline (§82-4a)**. Although the study lacks evaluations of several parameters that are normally conducted in a subchronic inhalation study, it is considered acceptable, when taken together with previously conducted 4-week subchronic whole-body exposure inhalation toxicity studies (MRIDs 40504818 and 40645903; HED Doc. No 012433), for determination of ChE and systemic toxicity NOAELs because the most sensitive endpoint, cholinesterase inhibition of blood and brain, was evaluated.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. A Flagging statement was not provided. This does not affect the integrity of the study. No adverse effects at exposure levels below subchronic inhalation toxicity NOAELs already in use for risk assessment purposes were identified in this study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Acephate
   Description: Technical, white solid
   Lot/Batch #: VJI-001TG-21 (Source #71047)
   Purity: 98.8% ai.
   Stability of compound: The expiration date of the lot provided was 9/9/00. Although stability information was not provided in the study report (stated to be on file with the Sponsor), the test material was stable in the dosing solution for the duration of the study.
   CAS #: 30560-19-1
   Structure:



2. Vehicle: distilled water (supplier, batch not indicated)

3. Test animals: Species: rat
   Strain: Crl:CD®(SD)IGS BR (outbred VAF/Plus®CD® (Sprague-Dawley derived albino)
   Age and weight at study initiation: Males, 6 weeks, 159 - 189 g;
   Females 8 weeks, 165-210 g
   Source: Charles River Laboratories, Kingston, NY

   Housing: Individually housed in stainless steel wire mesh cages (except for exposure

periods)
Diet: PMI Nutrition International Certified Rodent Diet No. 5002 (meal), *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions: Temperature: 20 to 23° C
Humidity: 25% to 52%
Air changes: not stated
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 2 weeks

B. STUDY DESIGN:

1. In life dates - start: December 15, 1999; end: January 13, 1999

2. Animal assignment

Animals were assigned by a computerized randomization program to the test groups in Table 1. The program assigned animals so that mean body weights in each group were equalized.

TABLE 1: STUDY DESIGN[a]

Test group	Daily Target (mg/L)	Daily Analytical Conc. (mg/L)	Daily Nominal Conc. (mg/L)[b]	MMAD μm	GSD	Rats/sex
Control	0.00	0	0	NA	NA	10
Low (LCT)	0.001	0.001064±0.000088	7.248±0.269	0.888	1.671	10
Mid (MCT)	0.003	0.003123±0.000249	6.810±0.387	1.080	1.739	10
High (HCT)	0.005	0.005550±0.000452	4.810±0.334	1.212	1.697	10

a = Data extracted from tables on p. 36 of study report, MRID 45134302.
b = Nominal concentrations were based on the aqueous mixtures used for test substance aerosolization.

Exposures were conducted over a 4-week period, 5 days/week, 6 hrs/day (total of 20 exposures). Animals were stagger-started in 4 groups of 20 rats (2-3/sex/group) over 4 consecutive days (December 15 through 18) for logistical reasons. Termination dates were selected accordingly (January 10 through 13).

2. Generation of the test atmosphere and description of the chamber: Exposures were conducted in 40 L cast aluminum and alloy ADG chambers with polycarbonate nose-only exposure tubes within $10M^3$ Harford glass and stainless steel exposure chambers.

To generate the chamber atmospheres, the test material was dissolved in distilled water to give 0.00% (controls), 0.01%, 0.06% and 0.10% solutions for the low, mid and high exposure concentration groups, respectively. Solutions were pumped into an

atomizer where they were mixed with houseline air to form an aerosol and then introduced into the top of the 40 L exposure chamber, where a second houseline air inlet diluted the aerosol to the appropriate test chamber concentration. Flowmeters and metering valves controlled the rate of houseline air into both inlets. Test atmospheres were then directed down to the inlet of the nose-only exposure chamber.

Chambers were operated dynamically under slight positive pressure at an airflow rate of 40.0 L/min to maintain a complete change of air every minute and 19% oxygen. Time to equilibrium (T99) was 4.6 minutes. Airflow rate and static pressure were recorded twice hourly throughout the exposure periods. Chamber temperature ranged between 19-23 °C and relative humidity between 13-52%. Chambers were exhausted through a coarse filter, a HEPA filter and activated charcoal. Animals were removed from the chambers no sooner than 30 minutes following termination of the exposure to allow for complete clearance of test material.

Test atmosphere concentration in each chamber was measured 4 times during each exposure. Samples were withdrawn from the breathing zone of each exposure chamber and collected onto glass fiber filters. Sample concentration was determined gravimetrically by dividing the difference between the pre- and post-sampling filter weights by the sample volume and by gas chromatography. Results of the concentration analyses are presented in Table 1, above. Mean daily chamber concentration values ranged as follows:

> LCT - 0.000963 to 0.001214 mg/L (most within 10% of target concentration);
> MCT - 0.002777 to 0.003537 mg/L (most within 10-15% of target) and
> HCT - 0.004899 to 0.007651 mg/L (concentrations slightly high on 9 exposure days but not considered to significantly affect the conclusions of the study).

Aerosol levels in each chamber were qualitatively monitored throughout the exposures using an aerosol sensor (RAM).

Nominal concentration was determined by weighing the generation apparatus containing test material before and after exposure. The difference in these weights was divided by the total volume of air (L) used during the exposure period to obtain nominal concentration. Results are provided above in Table 1. The study report stated that the differences between nominal and analytical concentrations are typical for this type of exposure and reflect deposition of the test material on surfaces within the chambers.

Particle size determination was performed using a TSI Aerodynamic Particle Sizer. Samples were collected once daily from each chamber for 20 seconds at 5 Lpm. Results are shown in Table 1, above.

Based on the analytical results, the chamber conditions and the concentrations of test material at each exposure level were within acceptable range of variation from the target concentration and the aerosol was respirable.

3. Rationale for exposure concentration selection - The exposure levels selected for this study were based on the results of a pilot inhalation exposure study, in which 5 females rats/exposure group were exposed to acephate at target concentrations of 0, 0.005, 0.025 or 0.050 mg/L by nose-only inhalation for 3 consecutive days (6 hrs/day). Analytical concentrations of the test material were (control to high concentration) 0.00, 0.0046, 0.0253 and 0.0525 mg/L. Results are summarized in Appendix L of the study report. Plasma and erythrocyte cholinesterase activities were analyzed on exposure days 1 and 3 (1 hr post-exposure) and on the day of termination (one day post-exposure) and brain cholinesterase (from the right and left brain halves separately) was analyzed on the day of termination (one day post-exposure).

No mortality, clinical signs of toxicity or body weight changes were observed.

Cholinesterase activities of blood and brain are shown below in Table 2:

TABLE 2: BLOOD AND BRAIN CHOLINESTERASE ACTIVITIES IN PILOT STUDY[1]

Mean Ambient Exposure Concentration in mg/L (Analytical)				
Sampling time	0.000	0.0046	0.0253	0.0525
PLASMA CHOLINESTERASE (IU/mL)				
Day 0 (1 hr post-exposure)	1.802	1.262* (-30)[2]	1.009* (-44.0)	1.016** (-43.6)
Day 2 (1 hr post-exposure)	1.676	1.164** (-30.5)	0.697** (-58.4)	0.666** (-60.3)
Day 3 (1 day post-exposure)	1.604	1.129** (-29.6)	0.743 (-53.7)	0.681** (-57.5)
ERYTHROCYTE CHOLINESTERASE (IU/mL)				
Day 0 (1 hr post-exposure)	1.710	1.280 (-25.1)	0.925** (-45.9)	0.815** (-52.3)
Day 2 (1 hr post-exposure)	1.260	1.040 (-17.5)	0.870** (-31.0)	0.640** (-49.2)
Day 3 (1 day post-exposure)	1.300	1.230 (-5.4)	1.305	0.760** (-41.5)
BRAIN CHOLINESTERASE (IU/mL)				
Day 3 (1 day post-exposure)				
Left brain	18.120	17.060 (-5.8)	13.370** (-26.2)	11.360** (-37.3)
Right brain	18.800	16.980** (-9.7)	13.250** (-29.5)	11.310** (-39.8)
Total brain	18.460	17.020* (-7.8)	13.310** (-27.9)	11.335** (-38.6)

1 Data extracted from Tables on pp. 310 and 311, Appendix L of MRID 45134302. Only females were tested. N = 5, all groups.

2 Values in parentheses indicate percent inhibition of activity relative to controls.

* Statistically significant, $p<0.05$ ** $p<0.01$

Plasma cholinesterase activity showed statistically significant reductions, generally

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dose-dependent, at all exposure levels at all time points. Erythrocyte cholinesterase activity was significantly decreased at all exposure levels on days 1 and 3 of exposure but only at the HCT on the day following the third exposure. Brain cholinesterase activity was significantly lower at the MCT and HCT. Activities in the left and right brain were similar and therefore only right brain was collected in the main study.

In addition to the pilot study, the previously conducted 4-week whole-body inhalation toxicity studies (MRIDs 40504818 and 40645903) were used as a basis for selection of the exposure concentrations.

4. Statistics - Body weight, body weight gain (calculated for study intervals), food consumption, organ weights, and blood and brain cholinesterase activities were analyzed statistically. For all parameters except organ weight data, the equality of variances was evaluated using Bartlett's test. Where variances were equal, one-way ANOVA using the F ratio was performed to assess significance among the means, followed by Dunnett's or Williams test to determine significant differences of treated groups from the controls. Where variances were unequal, the Kruskal-Wallis test, followed by Dunn's test or Shirley's test, was performed to identify significant differences of treated groups from the controls. For organ weight analysis, Bartlett's test was performed to evaluate equality of variances, followed by one-way ANOVA using the F ratio to assess significance among the means. Where significant differences were identified, Cochran and Cox's modified t-test was used to determine whether means were significantly different from controls. Statistical significance was identified at 1% for Bartlett's test and at 1% and 5% for all other tests.

C. METHODS:

1. Observations:

Animals were inspected cageside twice daily for visible signs of toxicity and mortality. During each exposure, animals were observed as a group at least once. A more detailed physical examination was conducted twice prior to initiation of exposures and weekly during the exposure period.

2. Body weight

Animals were weighed twice prior to initiation of exposures, weekly during the testing period and at the study termination (fasting weight) just prior to necropsy on the day following the final exposure.

3. Food consumption

Food consumption for each animal was determined weekly over a 7-day period

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starting 1 week prior to initiation of exposures. Mean daily diet consumption was calculated as g food/animal/day. Food efficiency was not calculated.

4. Ophthalmoscopic examination

Eyes were not examined during this study.

5. Hematology and clinical chemistry

Hematology and clinical chemistry parameters were not evaluated in this study.

6. Cholinesterase determinations

Blood - Plasma and erythrocyte cholinesterase activities were analyzed on exposure days 1 and 5 (within 1-hr post-exposure) and on the day following the 20th (final) exposure. Samples were collected into heparinized tubes from the orbital sinus of animals under light CO_2/O_2 anesthesia. Blood was separated into plasma and cellular components for analysis of cholinesterase activities.

Brain - At study termination, brains were removed and the right half homogenized immediately after dissection. A sample was analyzed for cholinesterase inhibition within 72 hrs.

All samples were analyzed using the Ellman method on a Hitachi 717 Chemistry Autoanalyzer. The remaining plasma and erythrocyte samples and brain homogenates were stated to have been stored frozen for up to 6 months in the event that additional testing was necessary. No further details of sample processing or storage were provided.

7. Urinalysis

Urinalysis was not conducted in this study.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination on the day following the final exposure. Adrenal glands, left half of the brain, epididymides, kidneys, liver, lungs, ovaries and testes were weighed. Histopathological examination was performed on control and high exposure group animals only for the following organs and tissues: kidneys, larynx, liver, lungs. nasopharyngeal tissues, trachea and any tissues with grossly visible findings.

II. RESULTS

A. Observations:

1. Toxicity - Some Group 4 (0.005550 mg/L) animals showed labored breathing during exposure in the final week of the study (25% to 33% of animals on days 24, 25 and 26). There were no other treatment-related clinical signs of toxicity reported during this study.

2. Mortality - Male #3009 (Group 3, 0.003123 mg/L) died after blood collection following the first exposure. Male #3011 replaced it. This death was not considered treatment-related because of the early time of death and lack of dose-response.

B. Body weight and weight gain:

Selected mean body weight and weight gain data are presented below in Table 3. Percent decreases relative to controls are indicated where appropriate.

TABLE 3: SELECTED MEAN BODY WEIGHT AND BODY WEIGHT GAIN[1]

Sex/parameter/ study day		Mean Ambient Exposure Concentration in mg/L (Analytical)			
		0.000	0.001064	0.003123	0.005550
Males					
Mean weight, g	-1	179	178	178	178
	6	228	219 (-3.9)[2]	220 (-3.5)	221 (-3.1)
	20	321	293 (-8.7)	299 (-6.9)	301 (-6.5)
	25	354	316** (-11)	328 (-7.3)	326 (-7.9)
Gain, g	-1 to 6	50	41 (-18)	42 (-16)	43 (-14)
	6 to 13	53	45** (-13)	45* (-15)	47 (-11)
	13 to 20	40	29** (-28)	34 (-15)	32 (-20)
	20 to 25	33	22** (-33)	29 (-12)	25 (-24)
	Cumulative[3]	175	138 (-21)	150 (-14)	148 (-15)
Females					
Mean weight, g	-1	190	197	198	197
	6	210	212	213	211
	20	238	235	239	234
	25	255	252	256	251
Gain, g	-1 to 6	20	15	15	13
	6 to 13	19	15	18	17
	13 to 20	9	8	8	6
	20 to 25	17	17	17	17
	Cumulative[3]	65	55 (-15)	58 (-11)	54 (-17)

1 Data extracted from Tables 3 through 6, MRID 45134302. N = 10, all groups.
2 Values in parentheses indicate percent decrease relative to the controls.
3 Cumulative weight gain calculated by reviewer and not analyzed statistically.
* Statistically significant, $p<0.05$ ** $p<0.01$

No treatment-related effects on body weight or weight gain were observed. At day 25, mean body weights (control to high concentration) were 354, 316, 328 and 326 g (males) and 255, 252, 256 and 251 g, females. The only statistically significant decrease in body weight occurred in LCT males during the last exposure week (-11% less than controls); at termination, the MCT and HCT groups were -7.3% and -7.9% less than controls, respectively. Body weight gain in males was significantly decreased in all intervals between days 6 and 25 at LCT and between days 6 and 13 at MCT. In females, mean body weights were comparable for all groups. Gain was slightly lower in all treated groups due to lower initial weight of control females, but there were no statistically significant differences and a dose-response was not observed. Although mean body weights/weight gains of the treated males and gains of the treated females were lower than the controls, there was no dose-response observed; therefore the decreases were not attributed to treatment. This conclusion is supported by the 4-week whole-body exposure inhalation toxicity study in the CD® rat (MRID 40504818; HED Doc. No. 012544), in

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which no decreases in body weight were observed at or below 0.0108 mg/L.

C. Food consumption:

No treatment-related effects on food consumption were observed. A statistically significant decrease in the LCT males during the final week of exposure (-15% less than controls) was considered sporadic.

D. Cholinesterase determinations:

Results of the blood and brain cholinesterase assays are presented below in Table 4:

TABLE 4: BLOOD AND BRAIN CHOLINESTERASE ACTIVITIES[1]

Sex/sampling time[2]	Mean Ambient Exposure Concentration in mg/L (Analytical)			
	0.000	0.001064	0.003123	0.005550
PLASMA, IU/mL				
Males				
Day 1	0.608±0.0996	0.554±0.0592 (-8.9)[3]	0.526±0.0293* (-13.5)	0.526±0.0514* (-13.5)
Day 5	0.567±0.0924	0.501±0.0723* (-11.6)	0.470±0.0436** (-17.1)	0.465±0.0590** (-18.0)
Termination	0.556±0.0987	0.483±0.0680 (-13.1)	0.484±0.0387 (-12.9)	0.510±0.0999 (-8.3)
Females				
Day 1	1.012±0.3152	1.140±0.2040 (+12.7)	1.182±0.3684 (+16.8)	1.156±0.2291 +(14.2)
Day 5	0.786±0.3012	0.960±0.1740 (+22.1)	0.989±0.2607 (+25.8)	0.910±0.1512 +(15.8)
Termination	1.245±0.3652	1.378±0.2883 (+10.7)	1.369±0.3663 (+10.0)	1.328±3216 +(6.7)
ERYTHROCYTE, IU/mL				
Males				
Day 1	1.236±0.1759	1.425±0.1756 (+15.3)	1.375±0.2000 (+11.2)	1.389±0.2043 (+12.4)
Day 5	1.220±0.1549	1.708±0.5789** (+40.0)	1.413±0.1796 (+15.8)	1.156±0.1499 (-5.2)
Termination	1.295±0.2226	1.710±1.0197 (+32)	1.305±0.5511 (+0.8)	1.123±0.3629 (-13.3)
Females				
Day 1	1.378±0.2620	1.293±0.1650 (-6.2)	1.272±0.2627 (-7.7)	1.281±0.2200 (-7.0)
Day 5	1.508±0.2949	1.333±0.2558 (-11.6)	1.185±0.3708* (-21.4)	1.056±0.1923** (-30)
Termination	1.300±0.2475	1.388±0.4266 (+6.8)	1.298±0.4229 (-0.2)	1.183±0.3478 (-9.0)
RIGHT BRAIN, IU/mL				
Males				
Termination	18.130±1.3415	18.250±0.9031 (+0.7)	16.330±1.1884**(-9.9)	15.545±1.4074** (-14.3)
Females				
Termination	17.715±0.9554	17.415±1.3292 (-1.7)	16.790±0.7909 (-5.2)	15.400±0.9724** (-13.1)

1 Data extracted from Table 6, MRID 45134302. N = 10, all groups.

2 All day 1 and 5 samples taken about 1 hr post-exposure and termination samples taken 1 day post-exposure.
3 Values in parentheses indicate percent change relative to controls.
* Statistically significant, $p<0.05$ ** $p<0.01$

In males, plasma cholinesterase activities showed statistically significant decreases at all exposure levels: on day 5 at 0.001065 mg/L and on days 1 and 5 at 0.003123 and 0.005550 mg/L. However, TB-I agreed with the conclusions of the study author that the decrease at day 5 in the 0.001064 mg/L group was marginal (-11.6%) and therefore not sufficient to establish an adverse effect level. This conclusion is supported by the findings of the previously conducted 4-week whole-body exposure inhalation toxicity study in the CD® rat (MRID 40504818), in which there was no inhibition of plasma cholinesterase activities at 0.00105 mg/L (plasma ChE LOAEL 0.0108 mg/L), although erythrocyte and brain cholinesterase were inhibited (erythrocyte/brain ChE NOAEL < 0.00105 mg/L). However, in this nose-only study, there were no erythrocyte or brain effects at 0.001064 mg/L. Statistically significant inhibition of plasma cholinesterase activities by 13.5% to 18% in the 0.003123 and 0.005550 mg/L exposure groups was observed at two time points (days 1 and 5) and was considered treatment-related in this study. No statistically significant decreases in erythrocyte activities at any time point, or in plasma activity at termination, were observed. Brain cholinesterase activity was significantly decreased below controls at termination at 0.003123 and 0.005550 mg/L (-9.9% and -14.3%, respectively).

Females appeared to be less sensitive to cholinesterase inhibition. No treatment-related inhibition of plasma cholinesterase was observed at any time point. A significant inhibition of erythrocyte cholinesterase activity was observed at 0.003123 and 0.005550 mg/L only on day 5 (-21.4% and -30%, respectively). Brain cholinesterase activity was significantly decreased at termination at 0.005550 mg/L (-13.1%).

E. Sacrifice and pathology:

1. Organ weight - No treatment-related changes in organ weights were observed. Several statistically significant differences from the control means were observed among treated groups but they were of small magnitude, did not show a dose-response and/or were related to decreased mean body weight in all of the treated groups (data not shown). Mean absolute liver weights of all treated male groups were significantly lower than controls (-9.5% to -11%) but a dose-response was not observed and relative weights were similar to controls. Mean relative brain weights of males were significantly increased in males at all exposure levels (due to the reduced body weights in all treated groups, but showed no dose-response. Significant increases in relative testes weights at low and high exposure groups (+11% and +10%) also showed no dose-response and were related to lower body weights in all treated groups and not to treatment.

2. Gross pathology - No treatment-related abnormal findings were observed upon gross

examination.

3. Microscopic pathology -

a) Non-neoplastic - No non-neoplastic lesions were identified as treatment-related.

b) Neoplastic - No neoplastic lesions were identified in the tissues that were examined.

III. DISCUSSION

A. This study was conducted to reevaluate the ChE NOAEL of 0.00507 mg/L (HCT; MRID 40645903) and erythrocyte/brain ChE LOAEL of 0.00108 mg/L (LCT; MRID 40504818) for inhalation exposure to acephate that were identified in 2 previously conducted 4-week whole-body exposure inhalation toxicity studies in the rat (see HED Doc. No. 012544 for reviews). Because these 2 earlier studies were conducted using whole-body exposure, which would be expected to give greater actual exposure levels due to deposition of test material aerosol on the animals (i.e., dermal plus inhalation exposures), this study was conducted to determine a NOAEL/LOAEL for inhibition of cholinesterase activities using nose-only inhalation exposure. The nose-only study was not designed according to the standard subchronic inhalation toxicity protocol and some parameters were not evaluated (only body weight and weight gain, food consumption, cholinesterase activities of blood and brain, gross examination at necropsy, organ weights and some tissues/organs were assessed). However, because (1) inhibition of cholinesterase activities is consistently the most sensitive parameter in the inhalation studies in rodents and (2) nose-only exposure is considered a more appropriate route of inhalation exposure than whole body for a test material aerosol that can deposit on the animals to a significant extent during exposures, this study is considered acceptable for regulatory purposes for determining a ChE NOAEL/LOAEL for inhalation exposure to acephate.

TB-I agreed with the conclusions of the study author that inhalation exposure of rats to acephate for 4 weeks resulted in inhibition of cholinesterase activity in the brain of males and also in erythrocytes of females at 0.003123 mg/L. Although the percent inhibition was relatively small (-9.9% less than controls), it was statistically significant ($p<0.01$), showed a dose-response and was also observed at the HCT in females.

In general, the cholinesterase inhibition observed in this study was relatively low. No statistically significant inhibition of plasma cholinesterase in females nor of erythrocyte cholinesterase in males was observed at any exposure level. At 0.005550 mg/L, brain cholinesterase activity was inhibited in both sexes by about -13 to -14%. Some animals at the highest exposure level also had respiratory distress (labored breathing) during the final exposure periods. At the lowest exposure concentration tested, 0.001064 mg/L, statistically significant decrease in plasma cholinesterase activity was observed on day 5 in males. Although a statistically significant decrease in plasma cholinesterase activity was observed

in males on day 5 at 0.001064 mg/L, it is not considered sufficient evidence of an adverse effect at that exposure level. It is noted that for some of the measurements (e.g., plasma cholinesterase in males and erythrocyte cholinesterase in females at at the mid and high exposure levels) inhibition observed at day 1 and/or 5 was not observed at termination. The reason for this (i.e. waiting 1 day post-exposure at termination to collect samples, or other cause) is not clear and was not discussed by the study author. Additionally, cholinesterase inhibition in plasma and erythrocytes was less pronounced in the main study at 0.005550 mg/L than in the pilot study at 0.0046 mg/L.

The ChE NOAEL for inhalation exposure is 0.001064 mg/L, based on inhibition of brain cholinesterase activity at the LOAEL of 0.003123 mg/L in males and possibly erythrocyte activity in females.

B. Study deficiencies: This study did not examine a number of parameters (ophthalmology, hematology, clinical chemistry, complete histopathology examination of organs and tissues) that are part of the standard protocol for Guideline §82-4a, subchronic inhalation toxicity study in the rodent. However, the study was intended to refine the systemic toxicity (ChE) NOAEL for subchronic inhalation exposure rather than to be a complete guideline study, since acceptable guideline inhalation studies were previously submitted. This study is therefore classified **Acceptable/guideline (§82-4a)** and may be used for regulatory purposes for determination of a NOAEL/LOAEL for inhalation exposure to acephate.

In the protocol deviation section of this study report, the study author noted that relative humidity was outside the desired range in the animal room (6 times, 25% to 52% instead of 30% to 50%) and in the exposure chamber (60 times, 13% to 54% instead of 40 to 60%) and chamber temperature was out of range by 1 degree on 1 occasion. These deviations are not considered to significantly affect the integrity of the study.

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Chemical:	**Acephate**
PC Code:	***103301***
HED File Code	**13000 Tox Reviews**
Memo Date:	**07/27/2000**
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