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

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APR 27 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 239-2471 - Acephate - Review of Rat 90-Day Feeding and Single Dose Dermal Studies Designed to Assess for Anticholinesterase Activity

TOX Chem. No.: 2A
Tox Project No.: 8-0567
Record No.: 215343

FROM: John Doherty 4/26/88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: William H. Miller, PM 16
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Registration Division (TS-767C)

THROUGH: Edwin Budd, Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Budd 4/26/88

The Chevron Chemical Company has submitted a 90-day oral subchronic dosing study designed to assess for potential anticholinesterase activity of acephate (in RBCs, plasma and brain) in response to a previous request by the Agency to provide such a study. A single dose dermal toxicity study was also submitted although the purpose for conducting this study was not provided.

TB Comments

These studies were reviewed and the following comments apply.

1. The rat 90-day subchronic oral study did not establish a NOEL for inhibition of brain AChE. The lowest dose level tested was 2 ppm (0.12 mg/kg for males and 0.15 mg/kg for females).

2. Both of these studies had rather large standard deviations for the group means of the individual rat RBC AChE, and plasma ChE activity determinations. These large standard deviations were considered to weaken the power of the statistical test used to evaluate the differences between the groups. This was particularly true for the rat dermal toxicity study. In this study, it was the conclusion of the testing laboratory that the plasma ChE (in males) and the RBC AChE (in females) was the most sensitive species. The oral toxicity study, and other data provided by the registrant, indicate that the brain enzyme is the most sensitive enzyme species. This discrepancy is difficult to explain in terms of route of administration and other experimental variables.

   The laboratory should be advised to refine their procedures for assessing AChE and ChE activities such that smaller standard deviations result. This is particularly applicable if the laboratory plans to do a 90-day dermal toxicity study as has been requested by the Agency.

Studies Reviewed

Study	Results	Classification
90-Day Oral - Sub-chronic Rat (AChE and ChE) Chevron Environmental Health Center #S-3068 December 30, 1987	NOEL < 2 ppm (brain AChE inhibited 7 to 9% in both sexes) For RBC AChE and plasma ChE: NOEL = 10 ppm LEL = 150 ppm	ACCEPTABLE [As a cholin-esterase in-hibition study.]
Single Dose Dermal - Rat (AChE and ChE determination) Chevron Environmental Health Center Study #S-2203 November 24, 1986	NOEL = 7.9 mg/kg for males LEL = 36.7 mg/kg males (inhibition of plasma ChE) At 153.9 mg/kg for females, inhibition of brain AChE and plasma ChE At 201 mg/kg for males, inhibition of brain AChE At 305.5 mg/kg for females, inhibition of RBC and brain AChE. [Note the above are the study reports conclusions.]	Not Classified

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Reviewed By: J.D. Doherty 4/26/88
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: E.R. Budd 4/26/88
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: 90-Day Subchronic Oral Study to Assess for Potential Anticholinesterase Effects in Rats

TOX Chem. No.: 2A
MRID No.: None

Accession No.: 405048-19

Test Material: Acephate (O,S-Dimethyl acetylphosphoramidothioate)

Synonyms: Orthene

Study No(s).: S-3068

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center, Richmond, California

Title of Report: The Cholinesterase Inhibition Potential of Acephate Technical (SX-1102) Following 4-, 9-, and 13-Week Dietary Administration in Male and Female Rats

Author(s): Brorby, G.P.; Rosenberg, D.V. (Study Director)

Report Issued: December 30, 1987

Conclusions:

NOEL < 2 ppm (Brain AChE inhibited 7 to 9% in both sexes)
[Note: 2 ppm = 0.12 mg/kg/day for males and 0.15 mg/kg/day for females.]

For RBC AChE and plasma ChE:

NOEL = 10 ppm
LEL = 150 ppm

Classification: Acceptable (as a cholinesterase inhibition study).

Special Review Criteria (40 CFR 154.7): No Special Review trigger.

4

Quality Assurance Statement:

A statement signed by Mr. B.M. Dowling attesting that five Quality Assurance reviews were conducted; three during the in-life phase of the study and two of the draft final report.

REVIEW

Experimental:

In this study the basic experimental design consisted of five groups of 60 rats [30 male and 30 female, Sprague-Dawley Crl : CDR (SD) BR obtained from the Charles River Laboratories Portage, Michigan Facility] were dosed with the nominal dietary concentrations of 0, 2, 5, 10, and 150 ppm. The rats were approximately 45 days old at the start of dosing. Subgroups I, II, and III consisting of 10 males and 10 females each from each dosage group were sacrificed at 4, 9, and 13 weeks after being initiated on the test diets.

The test material was acephate technical (O,S-dimethyl acetylphosphoramidothioate) and it was from Lot No. SX-1102. It was stated as being 98.2 percent pure but the impurities were not identified. The acephate was incorporated into the diets (Purina Certified Rodent Chow Meal No. 5002) by mixing acetone solutions with a Hobart mixing bowl. The diet containing 150 ppm of acephate was prepared first and the diets containing 10, 5, and 2 ppm were prepared by diluting the 150 ppm preparation with control (acetone-treated) diet. Fresh diets were prepared on the Monday of each week and stored frozen until use. The rats were offered fresh diets on Tuesday and Friday of each week. Samples of the test diets were taken to assure homogeneity of mixing and stability during freezing and for the 3- to 4-day time interval that the diets remained in the hoppers. The chemical analyses of the diets were performed and reported separately (in Appendix A of the report) by the Chevron Chemical Company, Residue and Metabolism Laboratory.

Results:

1. Dietary Analysis

The overall mean dietary acephate concentrations for the four test groups were analyzed to be 1.74, 4.29, 8.99, and 132 ppm (Table I of the study report) for the duration of the 13-week study. These values are 87, 85.8, 89.9, and 88 percent of the nominal dietary concentrations of 2, 5, 10, and 150 ppm. The mean weekly dietary concentration was determined by analyzing the sample concentration after being frozen for 1 or 4 days and after being in the hopper for 3 or 4 days and averaging out the mean value. From 6

to 17 percent of the acephate in the diets apparently decomposed over the 3- or 4-day interval in the hoppers.

2. Acephate Intake

The overall average acephate intake in mg/kg/day for the 13-week study is illustrated in the following table. These values were calculated by determining the body weight and the food consumption as well as the concentration of acephate in the food.

Nominal Dietary Level (ppm)	Acephate Ingested (In mg/kg/day)	
	Males	Females
2	0.12	0.15
5	0.28	0.36
10	0.58	0.76
150	8.90	11.48

Due to increases in the body weights of the rats, the mean daily intake of acephate was lower in the final weeks of the study by 48 percent in males and 35 percent in females when consumption data for week 13 were compared with week 1.

3. Mortality

No deaths resulted.

4. Signs of Toxicity

The rats were observed for signs of toxicity twice weekly (Tuesday and Friday). There was no elaboration as to specifically how the rats were observed for signs of toxicity. Thus, it is assumed by TB that only the more obvious changes in the behavior of the rats would have been noted. There is no mention in either the protocol or the study results to indicate that any special tests were made to assess for neurobehavioral toxicity responses.

No signs of toxicity were reported in the appearance or behavior of the test rats to indicate toxicity responses to treatment with acephate.

5. Body Weights

The rats were weighed on the first day of treatment and weekly thereafter. There were no changes in body weight

in either consistency or magnitude to indicate an effect of acephate.

6. Cholinesterase Determinations

[Note: The rats were sacrificed by exsanguination following anesthesia with sodium pentobarbital after 4, 9, and 13 weeks of dosing. A blood sample of about 1 mL was withdrawn from the descending aorta to be used for red blood cell (RBC) and plasma cholinesterase (ChE) assessment. The brain was excised and cut at the level of the foramen magnum and was further cut along the midline into right and left sides. The left side was prepared for AChE assay and the right side was frozen. The brain was prepared for analysis by homogenizing in buffer (pH 7.3 Trizma, HCl, NaCl, EDTA free acid, and Triton X-100) with a Polytron for 30 seconds, allowed to stand for 45 minutes and centrifuged at 30,000X g for 45 minutes. The supernatant was frozen and assayed later for AChE determinations.

The whole blood was centrifuged for 3 minutes at 8800X g to separate the plasma from the RBCs. The plasma was drained off the packed RBCs and frozen for future assay. The RBCs were resuspended in isotonic buffer and centrifuged again for 3 minutes at 8800X g. The packed RBCs, were lysed by adding 2% Triton X-100 and diluted with distilled water. It was not stated as to whether or not the lysed RBCs were frozen prior to assay.]

Brain, RBC and plasma ChE activities were measured by a method based on that of Ellman (Biochem. Pharmacol. 7:88-95, 1961). When brain or RBC enzyme activity was measured, acetylthiocholine was used as the substrate and the enzyme activity was defined as acetylcholinesterase (AChE). When plasma enzyme activity was studied, butyrylthiocholine was used as the substrate and the activity was defined as nonspecific ChE activity.

Two different analytical instruments were used to determine the enzyme activity. All assays for Subgroup I (4 weeks) and the RBC assays only for Subgroup II were performed on a Varian DMS 100 UV/Visible Spectrophotometer. The plasma and brain activities for Subgroup II and all activities for Subgroup III were performed on a COBAS/FARA Centrifugal Analyzer (Roche Diagnostics). This apparatus allowed for separate programs to test for brain, RBC, and plasma enzyme activities.

The utilization of two different instrument systems as was done in this study is not considered a good practice by TB. The raw data, however, do not indicate any

difference in the values for brain, RBC, AChE, or plasma ChE such that the use of the two different instrument systems compromised the study. The brain values for the controls, however, were higher for Subgroups II and III. It was also noted that there were large standard deviations for the group means, especially for the RBC and plasma enzyme activities, sometimes as high as about 50 percent of the mean value. Brain AChE standard deviations were more reasonable, being about 1 to 6 percent.

The brain AChE activity was calculated two ways. The first was International Units of ACh/gm of brain tissue. The second was specific activity, which was based on units of enzyme activity per mg of protein. This required the determination of brain protein per sample and is considered a more accurate assessment of brain activity. The results of determination of brain, RBC, AChE, and plasma ChE activities are as follows:

a. Brain AChE

The results of analyzing the brain for AChE activity are shown in the following table (reproduced from the study report).

Brain Specific Activity (% of Controls)

		4 Weeks	9 Weeks	13 Weeks
Males	2 ppm	99	97	93**
	5 ppm	93**	93**	90**
	10 ppm	89**	86**	84**
	150 ppm	54**	51**	48**
Females	2 ppm	98	91**	91**
	5 ppm	95*	90**	90**
	10 ppm	90**	85**	86**
	150 ppm	56**	47**	47**

*Significantly lower than controls ($p < 0.05$).
**Significantly lower than controls ($p < 0.01$).

This study shows that for group 2 (2 ppm) males brain AChE activity is depressed (-7%) at 13 weeks and for females it is depressed at both 9 weeks (-9%) and 13 weeks (-9%). All other groups were statistically significantly depressed at all times. For example, group 3 (5 ppm) was also depressed

from 7 to 10 percent in the males and 5 to 10 percent in the females. Group 4 (10 ppm) male rats were depressed 11 to 16 percent and females were depressed 10 to 15 percent. The high-dose group (150 ppm) was depressed about 44 to 53 percent for both males and females.

Based on these determinations, no NOEL is established for inhibition of brain AChE following dietary administration of doses as low as 2.0 ppm.

[Note: These values are based on the specific activity expressed as activity per mg of protein which is considered a more reliable estimate of the activity. When the data are expressed as activity/gm of brain (wet weight of brain), the females at week 4 dosed with 2 ppm also show a statistically significant decrease in AChE, but this could be accounted for by two rats which had low protein content in the sample.]

b. RBC AChE

The results of analyzing RBC for AChE is illustrated in the following table (reproduced from the study report).

RBC AChE Activity (% of Controls)

		4 Weeks	9 Weeks	13 Weeks
Males	2 ppm	110	103	105
	5 ppm	103	104	113
	10 ppm	96	101	101
	150 ppm	52**	68*	78
Females	2 ppm	96	110	97
	5 ppm	100	93	92
	10 ppm	111	82	90
	150 ppm	70	56**	55**

*Significantly lower than controls ($p < 0.05$).
**Significantly lower than controls ($p < 0.01$).

These data support a NOEL of 10 ppm and an LEL of 150 ppm for inhibition of RBC AChE.

c. Plasma ChE

The results of analyzing the plasma for ChE activity is illustrated in the following table (reproduced from the study report).

Plasma BChE Activity (% of Controls)

		4 Weeks	9 Weeks	13 Weeks
Males	2 ppm	103	102	98
	5 ppm	98	94	88
	10 ppm	103	104	81
	150 ppm	72	74	64
Females	2 ppm	90	90	71
	5 ppm	81	94	85
	10 ppm	81	87	77
	150 ppm	73	54	57**

*Significantly lower than controls ($p < 0.05$).
**Significantly lower than controls ($p < 0.01$).

There is apparently little marked effect of acephate on plasma ChE since only the high-dose group females reached statistical significance.

[Note: For both RBC AChE, and plasma ChE the high standard deviations obtained when the averages for the 10 rats in each group were assayed may have weakened the statistical analysis of the data. For example, if the standard deviations were smaller, as would result from better precision among the trials for each rat, other groups may have shown statistical significance.]

7. Necropsy

No test chemical effects were noted following a "complete necropsy examination (including examination of the external surface of the body, all orifices, and internal examination of the cranial, thoracic, and abdominal cavities)." The tissues were not further analyzed microscopically.

Conclusion:

This study does not demonstrate a NOEL for inhibition of brain AChE. Significant inhibition of brain AChE (7 to 9%) was evident in both males and females at weeks 9 and 13 at a dietary level of 2 ppm (or more specifically 1.74 ppm or 0.12 mg/kg/day in males and 0.15 mg/kg/day in females).

At higher dietary levels (150 ppm), RBC AChE is inhibited (NOEL = 10 ppm). Plasma ChE determinations varied widely and only one subset showed statistical significance (females after 13 weeks of dosing with the 150 ppm diet, NOEL = 10 ppm).

TB recognizes that this study was designed to assess for potential inhibition of ChE and considers that the study is ACCEPTABLE for this purpose in as far as experimental design and execution is concerned. Thus, the NOEL of < 2.0 ppm (or < 0.12 or 0.15 mg/kg/day) can used for regulatory purposes such as setting of the ADI, etc.

Lastly, although this study is considered to be ACCEPTABLE, TB recognizes that the large standard deviations for RBC AChE, and plasma ChE assays may have decreased the power of the statistical tests used to determine differences among the dose groups.

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Reviewed By: J.D. Doherty 4/26/88
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: E.R. Budd 4/26/88
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Single Dose Dermal Cholinesterase Evaluation

TOX Chem. No.: 2A
MRID No.: N/A

Accession No.: 405048-20

Test Material: Acephate (O,S-Dimethyl acetylphosphoramidothioate)

Synonyms: Orthene

Study No(s).: S-2283

Sponsor: Chevron Chemical Company

Testing Facility: Chevron Environmental Health Center, Richmond, California

Title of Report: The Cholinesterase Inhibition Potential of Acephate Technical (SX-1102) Following Dermal Administration in Male and Female Rats

Author(s): Brorby, G.P.; Rosenberg, D.V. (Study Director)

Report Issued: November 24, 1986

Conclusions:

NOEL = 2 mg/rat (7.9 mg/kg for males). At the next higher level (36.7 mg/kg for males) and above there was statistically significant inhibition of plasma ChE. At 153.9 mg/kg and above in females there was inhibition of both brain AChE and plasma ChE. At the highest dose levels tested (201 mg/kg for males and 306 mg/kg for females) there was inhibition of brain AChE in males and RBC AChE in females. The above are the conclusions of the testing laboratory.

Classification:

No classification assigned (no stated purpose for conducting the study was provided) and the study is not a CORE study.

Special Review Criteria (40 CFR 154.7): None

Quality Assurance Statement:

A statement signed by R.W. Dowling indicating that Quality Assurance reviews were made on two occasions.

REVIEW

Experimental:

In this study, the basic experimental design consisted of dosing five groups of rats (each group had five male and five female Sprague-Dawley Crl:CD CSD) BR rats obtained from the Charles River Portage, Michigan Facility) with either 0, 2, 10, 30, or 60 mg/rat of acephate (O,S-dimethyl acetylphosphoroamidothioate) applied dermally as a single dose. Young adult rats (52-day-old males and 59-day-old females) were used such that the dosage levels tested were as presented in the following table:

Group	mg/rat	Males (mg/kg)	Females
A	0	0	0
B	2	7.9 ± 0.4	9.4 ± 0.5
C	10	36.7 ± 1.5	51.7 ± 2.2
D	30	107.0 ± 6.2	153.9 ± 11.0
E	60	201.0 ± 11.5	305.5 ± 15.1

The rats were prepared for treatment by clipping the fur on the day of dosing. One hour before application, the area to be treated was shaved free of hair and washed with soap and water. A neoprene rubber template was glued to the back of the rat such that a 4 x 6.25 cm application site was exposed. The test material (acephate dissolved in a 0.1% Tween 80 (w/v) in distilled water was applied in a volume of 0.2 mL to the prescribed area and spread with the assistance of a glass capillary tube. There was no indication that the application site was further covered to prevent loss of the test material. The rats, however, were fitted with Queen Anne collars which helped to prevent them from ingesting the test material.

The rats were reported to be examined 4 times on the day of dosing and once a day until sacrifice. The rats were sacrificed 72 hours after the initial treatment by anesthesia with sodium pentobarbital. A blood sample of 1 mL was withdrawn from the descending aorta and separated into plasma and RBCs. The brain was excised and cut into right and left halves. The left half was prepared for assay for AChE activity.

AChE and ChE enzyme activities were assessed in the RBC, plasma or brain using a method based on that of Ellman et al. (Biochem. Pharmacol. 7:88-95, 1961). The activity in brain or RBC was assessed using acetylthiocholine and the enzyme activity was defined as AChE. When plasma was assessed, butyrylthiochol-

ine was used on the substrate and the activity was defined as BChE. The brain activity of AChE was expressed as specific activity per mg of protein.

Results:

1. Mortality--No rats died as a result of treatment.

2. Behavioral Symptoms/General Condition--No obvious signs of toxicity developed. Some indications of "chromodacryorrhea" and "chromorhinorrhea" as well as "anogenital" discharge were noted but these were in both control and treated groups.

3. Body Weight--No effect on body weight was noted.

4. Gross Pathology and Histopathology--No compound related effects were noted.

5. AChE and ChE Determinations in RBCs, Brain, or Plasma--[Note: Tables 5 and 6 attached, reproduced from the study report, present the results of analyses for AChE and ChE.]

   a. RBC AChE Effects

      Large standard deviations were noted for the RBC AChE determinations when the average was determined for the five females and five male rats. In the case of the high-dose group males (only four rats), a standard deviation of about 50 percent resulted. The high standard deviations may have weakened the statistical power of the tests used to determine statistical significance (Dunnett's two-sided test).

      The only group showing statistical significance was the high-dose group females (59% inhibition). The other female dosed groups showed inhibition for the 10 (26%) and 30 (40%) mg/rat dose groups but these data did not demonstrate statistical significance.

      Among the males, none of the dosed groups showed statistical significance. There was noted, however, 21, 33, and 30 percent inhibition for the 10, 30, and 60 mg/rat dosed groups.

A NOEL of 30 mg/rat is implied for inhibition of RBC AChE.

b. Brain AChE

The standard deviations for the average specific activity of the brain AChE determinations were also large (16 to 40% for males; 7 to 35% for females). [Note in the 90-day feeding study the standard deviations were around 10% or less.]

Brain AChE for both the male (31% inhibition) and female high-dose group (51% inhibition) and female group receiving 30 mg/rat (39% inhibition) were statistically significantly depressed. The other male groups were also depressed by -3, -15, and -28 percent to give the pronounced appearance of a dose response for increased inhibition with increasing dose level. Among the females there was also a pronounced appearance of a dose response with the two lowest test dose levels being -7 and -10 percent depressed, respectively.

Based on reported statistical significance, a NOEL of 10 mg/rat is supported by these data. It should be clearly indicated that trends for inhibition of brain AChE are evident in both sexes, at lower doses.

c. Plasma ChE

Statistically significant decreases in plasma ChE were evident in males at all dose levels above 2 mg/rat. There was 18 percent inhibition also apparent at the 2 mg/rat dose level. Among females, statistically significant depression in this enzyme were noted at dose levels above 10 mg/rat. There was also noted 19 percent depression of this enzyme's activity at both of the lower dose levels for this sex.

Overall, the testing laboratory asserts that the NOEL for this study is 2 mg/rat (7.9 mg/kg for males) based on the finding of statistically significant inhibition of plasma ChE at 10 mg/rat and above.

6. The study report also attempts to determine the ID_{50} (derma dose of acephate that will produce 50% inhibition) for each enzyme species by using linear regression analysis. The

15

report concludes that the plasma enzyme would be the most sensitive species in males. In the males, the brain enzyme would be more sensitive than the RBC enzyme. In females the RBC enzyme would be the most sensitive species followed by the plasma enzyme and then the brain enzyme.

TB notes that in both sexes following oral administration of acephate, the brain enzyme was clearly the most sensitive species. TB does not consider either the ID_{50} estimations or the relative sensitivities of the enzymes as discussed in the study report to be useful in assessing the potential susceptibility of AChE and ChE to acephate.

Conclusion:

Since there was no specific purpose indicated for conducting this study and it is not a CORE study, no classification will be assigned.

TB notes that the conclusions of the study report regarding the most sensitive species of enzyme being plasma ChE or RBC AChE rather than the brain AChE is difficult to accept. Other studies have shown that the brain AChE is a more sensitive enzyme species than the plasma ChE particularly following oral administration of acephate. It is very difficult to accept that the difference in sensitivity of the enzyme species is due to route of administration (oral versus dermal) and other experimental variables. The large standard deviations noted for the means of the groups may have so weakened the power of the statistical tests that a true picture of the potential AChE of acephate by dermal exposure (single dose) was not attained by this study. Indeed the trends noted for decreased brain AChE activity at the lower dose levels would suggest that the enzyme is being inhibited by acephate at these dose levels.

The testing laboratory should be advised to refine their methods for assessing RBC AChE and plasma ChE such that smaller standard deviations result.

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Pages 17 through 18 are not included.

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