DATA EVALUATION RECORD

ACEPHATE

Study Type: OCSPP Non-Guideline; Cholinesterase Inhibition and Pharmacokinetics in Humans

EPA Contract No. 68HERC22D0017
Task Assignment Form No. 5540-3-008 (MRID 45388301)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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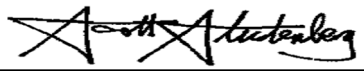
Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 10/14/2022
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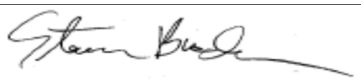
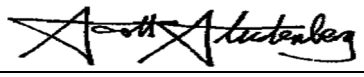
Quality Assurance: Steven Brecher, Ph.D.	Signature: Date:	 10/25/2022
Project Manager: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 10/25/2022

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DATA EVALUATION RECORD

STUDY TYPE: Cholinesterase Inhibition and Pharmacokinetics - Human; OCSPP Non-Guideline.

PC CODE: 103301; 101201 **DP BARCODE:** D465948
TXR#: 0058561 **SUBMISSION:** 1086785

TEST MATERIALS (PURITY): Acephate technical, 99.0%

SYNONYMS: *O,S*-dimethyl *N*-acetylphosphoramidothioate; methamidophos (*O,S*-dimethyl phosphoramidothioate [metabolite])

CITATIONS: Freestone, S., and McFarlane, P. (2001) A single oral dose study with acephate technical in humans – Report Amendment 2. Inveresk Research, Elphinstone Research Centre, Tranent, Scotland. Laboratory Project ID: ICR 013072, March 23, 2001. MRID 45388301. Unpublished.

SPONSOR: Valent USA Corporation, 1333 North California Blvd., Suite 600, P.O. Box 8025, Walnut Creek, CA

EXECUTIVE SUMMARY: In a non-guideline, double blind, placebo controlled, pharmacokinetic and cholinesterase (ChE) inhibition study (MRID 45388301), fifty (40 male; 10 female) human volunteers were administered a single oral dose of acephate technical (Lot No. 80121; purity 99.0%) in a hard gelatin capsule at 0.35 (7 males), 0.70 (7 males), 1.00 (7 males and females, respectively), or 1.25 mg/kg (7 males). Three male volunteers at each treatment level were administered lactose in a hard gelatin capsule (placebo). An additional 3 female subjects were administered placebos for the 1.00 mg/kg treated group. All subjects were housed in a clinical setting through 72 hours post-dose administration. Clinical signs were monitored up to 336 hours (Day 14) post-dose and vital signs were monitored up to 24 hours post-dose. Blood samples for hematology and clinical chemistry evaluations and urine samples for clinical urinalysis were collected at 24 hours post-dose. Blood samples for plasma and red blood cell (RBC) ChE activity determinations were collected up to Day 14 post-dose, in addition to multiple pre-dose collections to determine baseline activity values. Additional blood samples were collected up to Day 14 post-dose for evaluation of systemic acephate and methamidophos (metabolite) exposure. Urine collections also were conducted through 48 hours post-dose for determination of excreted acephate and methamidophos concentrations in urine.

There were no treatment-related effects on clinical signs, vital signs, or clinical pathology findings.

The acephate plasma concentration vs. time profiles were consistent with oral dose administration with no evidence of a significant lag phase. Mean time of maximum plasma concentration (T_{max}) estimates ranged from 1.3-2.7 hours. Acephate concentrations declined rapidly up to 24 hours post-dose, with mean elimination half-life ($t_{1/2el}$) estimates of 4.4-5.4 hours. Mean clearance (CL/F) estimates were generally slow, with values of 77-105 mL/hour/kg. Overall systemic exposure demonstrated generally dose-related increases (slightly greater than dose proportional). There were no sex-related differences in pharmacokinetic parameters at 1.00 mg/kg (the single dose level in female subjects).

Plasma concentration data were sparser for the metabolite, methamidophos, but valid T_{max}, maximum plasma concentration (C_{max}), and area under the curve (AUC_{0-t}) estimates were determinable at ≥0.70 mg/kg acephate, with valid estimates for the remaining parameters at ≥1.00 mg/kg acephate. Mean T_{max} estimates ranged from 3.3-4.3 hours. Methamidophos concentrations declined rapidly up to 12 hours post-dose, with mean $t_{1/2el}$ estimates of 6.7-8.3 hours. Mean CL/F estimates were rapid, with values of 3377-4439 mL/hour/kg. Overall systemic exposure demonstrated generally less than dose-proportional increases. There were no sex-related differences in pharmacokinetic parameters at 1.00 mg/kg.

The total recovery of acephate and methamidophos (as acephate equivalents) in urine through 48 hour post-dose ranged from 25% to 62% in males and 12% to 51% in females, with most of the recovery occurring within the initial 12-hour post-dose period. The fractions of the administered acephate dose recovered as methamidophos were <1% AD and ≤2% of the total recovered dose and were independent of dose or sex. Urine concentrations of methamidophos (systemic exposure) generally decreased in parallel to acephate at ≥1.00 mg/kg acephate and accumulation of methamidophos would not be expected.

Mean plasma ChE activities showed statistically significant decreases (as compared to baseline values) of 11% in low dose males (0.35 mg/kg) at 12 hours post-dose, and 9-13% at 12, 24, 48, and 72 hours post-dose in the high dose (1.25 mg/kg) males. Non-statistically significant decreases were observed at 0.70 (↓10%) in males at 1 hour and 12 hours post-dose as well. Mean plasma ChE activities were decreased by 10-13% at 8, 12 and 24 hours post-dose in females (1.00 mg/kg), these changes were also statistically significant.

For RBC ChE activities, decreases of 7% and 11% were noted in the 1.25 mg/kg males and the 1.00 mg/kg females, respectively, at 12 hours post-dose (both showing statistical significance). A non-statistically significant decrease of 8% was also observed at 8 hours post-dose in the 1.25 mg/kg males. A 12% non-statistically significant decrease in mean RBC ChE was observed in the 1.00 mg/kg females at 72 hours post-dose, without statistical significance.

In male subjects, statistically significant decreases in both plasma and RBC ChE activities were noted only at 12 hours post-dose in the 1.25 mg/kg group. In female subjects, statistically significant decreases in both plasma and RBC ChE activities were also noted only at 12 hours post-dose in the 1.0 mg/kg group. All changes in plasma ChE were within the variability of baseline values at each dose level (with the exception of the 13% change at 12-hours in the 1.25 mg/kg males compared to an 11% CV at baseline at this dose), as well as within the variability of

placebo group controls. There was less variability in the results for RBC ChE, however, results were still within variability of baseline values for males, and slightly outside for females (11% change at 1.0 mg/kg compared to baseline CV of 9%). Due to the variability of the data and no clear dose response for AChE inhibition, there is currently no intention to use these AChE data for risk assessment purposes, including future physiologically-based pharmacokinetic (PBPK) modeling.

Analytical methods for the determination of acephate and methamidophos in human blood and urine were described and validated. Storage and stability were also verified. The analytical methods (No. 6696 for human blood/plasma, and No. 6695 for human urine), were validated for the determination of acephate and methamidophos at a concentration range of approximately 10 to 1,000 ng/mL.

There were several results that fell outside of the method validated range of 10 to 1,000 ng/mL for both human blood/plasma and urine. Mean urine concentration data for acephate and methamidophos were outside of the method validated concentration range at the with exceedances of 1,000 ng/mL for acephate at 0-12 hours (all dose levels, both sexes), 12-24 hours (all dose levels, both sexes), and 24-48 hours (1.00 and 1.25 mg/kg dose levels (males)). For methamidophos, concentrations were below the limit of quantification of 10 ng/mL at 24-48 hours for the 0.70 mg/kg (males) and 1.0 mg/kg (females). Mean plasma concentration data for acephate and methamidophos were also outside of the method validated concentration range of 10-1000 ng/mL at several dose levels and timepoints. For acephate, mean plasma concentrations were above 1000 ng/mL at 1.0 and 1.25 mg/kg in males at 1, 2, and 4 hours post-dose, and at 1.0 mg/kg in females at 2 and 4 hours post-dose. For methamidophos, mean plasma concentrations were below 10 ng/mL at 0.70 mg/kg (2, 8, and 12 hours post-dose), 1.0 mg/kg (1 and 12 hours post-dose), and 1.25 mg/kg (1 and 12 hours post-dose) in males, and at 1.0 mg/kg (1 and 12 hours post-dose) in females.

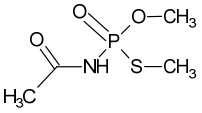
This study is classified **acceptable/non-guideline**. Although there were data that fell outside of the method validated range for concentrations of acephate and methamidophos in plasma and urine, information from the study is still considered useful for improving predictability of future PBPK modeling.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compounds:

Non-Radiolabeled TGAI:	Acephate technical (two portions)
Description:	White powder
Lot No.:	80121
Purity:	99.0%
CAS No. of TGAI:	30560-19-1
Expiration/storage:	August 5, 1999 and July 21, 2000/−20°C
Structure:	

2. Placebo: Lactose (J.M. Loveridege [Batch No. 28811] and Thornton and Ross [Batch No. 18TJ])

3. Subjects:

Species:	Human
Sex/Age:	Males and females/ 18-50 years old (study criterion)
Age/weight at administration:	Placebo: 18-45 years/61.1-81.6 kg (12 males) 0.35 mg/kg: 27-41 years/62.7-94.8 kg (7 males) 0.70 mg/kg: 24-41 years/60.5-81.8 kg (7 males) 1.00 mg/kg: 29-46 years/70.8-83.7 kg (7 males) 1.25 mg/kg: 20-48 years/54.7-78.9 kg (7 males) Placebo: 26-37 years/59.5-71.1 kg (3 females) 1.00 mg/kg: 20-46 years/51.3-81.6 kg (7 females)
Source:	Volunteers registered at ICR and screened within 21 days of dosing
Housing:	Volunteers were housed in the treatment clinic for the initial 72 hours post-dose.
Diet:	A standard breakfast was provided prior to the single dose administration. Alcohol and caffeine consumption were not permitted during the initial 72 hours post-dose.

4. Selection criteria: Inclusion/exclusion criteria, pre-study screening parameters, restrictions, and actions resulting in removal from the study were detailed in the study report (MRID 45388301). The study protocol and volunteer information were submitted to the Independent Ethics Review Committee of Inveresk Research for review and approval prior to study initiation. The main criteria for inclusion were healthy male or female volunteers between the ages of 18-50 years old, additional details can be found in Appendix III of this DER.

5. Preparation of study capsules: The test item or placebo were administered in size zero hard gelatin capsules. The required doses were prepared by direct weighing of the bulk acephate technical or lactose into capsules based on the screening weights of each individual study subject. Acephate technical reference capsules analyzed at the end of the study contained the stated amounts of the test item and placebo capsules did not contain any test item. No decomposition of the test item was observed during the course of the study.

B. STUDY DESIGN AND METHODS

1. Group arrangements: Study design details are presented in Table 1. The study report indicates that the objective of the study was to determine the highest no observed effect level (NOEL) and/or the lowest observed effect level (LOEL) of acephate causing a slight inhibitory effect on blood cholinesterase concentration in human volunteers. The NOEL is defined as the highest dose tested at which no inhibition of plasma and red blood cell cholinesterase activity occurs. The study was conducted as a double-blind, placebo-controlled study. The body weight for each subject was determined prior to capsule preparation. Dose administration was staggered with each treatment level evaluated in a single subject prior to administration to the remaining subjects per level (n = 6). The group of female volunteers were evaluated in the final session (Session 6) with all seven subjects as the 1.00 mg/kg dose level had been previously evaluated in male volunteers. The in-life portion of the study was initiated on June 9, 1999 and completed on August 23, 1999. A subject number was assigned for all subjects who qualified for the study in accordance with the inclusion/exclusion criteria. Subject numbers were assigned sequentially using a computer generated randomization starting at 001.

TABLE 1. Dosing and sampling schedule after a single oral dose of acephate technical up to 1.25 mg/kg to human volunteers. [a]

Session	Sex	Number of subjects					Sampling Schedule
		0 mg/kg	0.35 mg/kg	0.70 mg/kg	1.00 mg/kg	1.25 mg/kg	
1	Male	1	1				Baseline plasma and red blood cell (RBC) cholinesterase concentrations were determined three times from Day −10 to −3, as well as Days −2, −1, and 30 minutes prior to dose administration. Blood samples were collected post-dose at 1, 2, 4, 8, 12, 24, 48, and 72 hours (in clinic) and on Days 7 and 14 post-dose. Urine samples were collected from −12-0 hours pre-dose and 0-12, 12-24, and 24-48 hours post-dose.
2	Male	2	6	1			
3	Male	3		6	1		
4	Male	3			6	1	
5	Male	3				6	
6	Female	3			7		

a Data were obtained from Table 1 on page 26 and pages 38-42 of MRID 45388301.

2. Dosing, safety evaluations, and sample collection

a. Dose selection: Each dose was evaluated for safety in a single subject (lead dose design) prior to administration to the remaining volunteers for that dose level and evaluation of the next highest dose level. The acephate dose levels were selected based on previous animal and human studies with acephate, including rat metabolism, subchronic and chronic studies in rats, subchronic study in primates, subchronic oral study in humans, a production plant worker exposure study, genetic toxicity studies, acute and subchronic neurotoxicity studies in rats, delayed neurotoxicity study in hens, carcinogenicity studies in rats and mice, and reproductive toxicity studies.

b. Dosing: The capsules (placebo or test item) for each dose session were prepared based on the individual body weight of each scheduled test subject. On the morning prior to

administration, the test subjects were fed a standard breakfast. The subjects were administered their respective capsules approximately five minutes after completion of breakfast with 150 mL water to aid swallowing of the capsule.

c. Safety evaluations: Blood pressure and heart rate was determined pre-dose and at 2, 4, 8, and 24 hours post-dose. Oral temperature was determined pre-dose and at 2, 4, and 24 hours post-dose. Electrocardiography (ECG) was determined pre-dose and 2, 4, 8, and 24 hours post-dose by 12-lead ECG. Single-channel continuous ECG monitoring was also conducted from 30 minutes pre-dose to 4 hours post-dose.

d. Sample collection

i. Blood: Blood collection for hematology and clinical chemistry evaluations was conducted pre-dose (30 minutes prior to dose administration) and at 24 hours post-dose. Samples were collected into EDTA-coated (3 mL) or heparinized (5 mL) tubes, respectively.

Baseline plasma and red blood cell (RBC) cholinesterase (ChE) concentrations were determined from blood collections (4.5 mL) at pre-screening, three times from Day −10 to −3, and Days −2, −1, and 30 minutes prior to dose administration. Blood samples were collected at 1, 2, 4, 8, 12, 24, 48, and 72 hours (in clinic) and on Days 7 and 14 post-dose. Daily blood samples (excluding all samples on Day 1, except the 1 hour post-dose collection) were collected in the morning and at approximately the same time each day. The blood samples were collected into EDTA and centrifuged to separate the plasma and RBC fractions. Samples up to 4 hours post-dose on Day 1 were stored at −20°C prior to transfer on wet ice for plasma/RBC ChE analyses that same day. Further samples were stored at −20°C overnight.

Additional blood samples (10 mL) were collected into lithium heparin at 0 hours (pre-dose) and at 1, 2, 4, 8, 12, 24, 48, and 72 hours (in clinic) and on Days 7 and 14 post-dose, centrifuged to harvest plasma, and stored at approximately −80°C for analysis of acephate and methamidophos (metabolite) concentrations.

Repeat samples were collected from four subjects, whose initial RBC ChE measurements were >20% from their baseline value (no other explanation provided):

- Subject 028 (Male; 1.25 mg/kg dose) – 72 hour RBC ChE measurement repeated
- Subject 036 (Male; placebo) – 48 hour RBC ChE measurement repeated
- Subject 038 (Male; placebo) – 72 hour RBC ChE measurement repeated
- Subject 043 (Female; 1.0 mg/kg) – 8 hour RBC ChE measurement repeated

ii. Urine: A collection of urine for clinical urinalysis was conducted at 24 hours post-dose. Urine samples for analysis of acephate and methamidophos (metabolite) concentrations were collected from −12-0 hours pre-dose and 0-12, 12-24, and 24-48 hours post-dose. Total urine volume was measured for each collection and a 20-mL portion was retained at approximately −80°C.

3. Sample analysis

a. Plasma and RBC ChE: Plasma and RBC ChE concentrations were determined by the method of Ellman (1961)[1].

b. Acephate and methamidophos - plasma: Acephate and methamidophos concentrations in human plasma were determined by gas chromatography with flame photometric detection after dilution with acetone containing polyethylene glycol (PEG). The method was validated over a concentration range of 10-1000 ng/mL. The limit of detection (LOD; 3× background) was 6 ng/mL for acephate and 1.5 ng/mL for methamidophos. The limit of quantification (LOQ) was 10 ng/mL for both compounds.

c. Acephate and methamidophos – urine: Acephate and methamidophos concentrations in human urine were determined by gas chromatography with flame photometric detection after dilution with acetone containing polyethylene glycol (PEG). The method was validated over a concentration range of 10-1000 ng/mL. The LOD was 7 ng/mL for acephate and 4 ng/mL for methamidophos. The LOQ was 10 ng/mL for both compounds.

4. Analytical methodology: The gas chromatography system consisted of a Hewlett Packard (HP) 6890 plus gas chromatograph (GC), HP 7683 injector and autosampler, and HP flame photometric detector with an RTX 200, 30 m × 0.53 mm i.d. 1.0 µm film thickness (Restek) analytical column. The GC system conditions were as follows:

Temperatures:	Oven:	Initial:	120°C hold for 0 min
		Rate:	20°C.min^{-1}
		Final:	250°C hold for 1.5 min
	Injector:	230°C	
	Detector:	250°C	
Gases:	Detector:	Hydrogen	100 ml.min^{-1}
		Air	100 ml.min^{-1}
		Aux (N_2)	50 ml.min^{-1}
	Carrier:	Hydrogen	10 ml.min^{-1}
Purge:	Mode:	Splitless	
	Time:	0.5 min	
	Flow:	3.0 ml.min^{-1}	
Injection Volume:	5 µl (a packed injection liner should be used)		
Data Handling:	LabSystems Vax Multichrom 2 Version 2.0		

Data obtained from page 1124, Appendix T of MRID 45388301.

5. Statistical methods: With a ratio of active subjects relative to placebo subjects of 2.33, a total of ten subjects (7 active and 3 placebo) were required per dose level to achieve 80% power to detect a difference of 20% change from baseline ChE concentrations. A between

1 Ellman, G.L. (1961) *Biochem. Pharmacol.* 7:78.

subject standard deviation (SD) of 8.7 and a 5% significance level were used for this calculation. Therefore, a total of 50 subjects (10 per dose level) were enrolled in the present study.

The objective of the statistical analyses was to investigate the clinical tolerability and safety of the test material. Statistical analyses were only conducted on the plasma and RBC ChE concentration data. Mean concentration (± SD) were reported at each time point, including the change from baseline. The baseline value was determined as the mean of all available pre-dose values, except the initial screening value.

The percentage change from baseline for plasma and RBC ChE concentration values were analyzed with a repeated measures analysis of variance (ANOVA), including dose level, time point, and dose level × time point interaction. The subject was included as a random effect. A test for linear trend with dose was conducted at each time point separately by using a linear contrast (data from males only). In addition, pairwise comparisons between placebo and each dose level were conducted for each time point with Student's t-distribution by using the error variance from the ANOVA. At each time point, if the test for linear trend was significant ($p<0.05$), the pairwise comparisons were not adjusted for multiple comparisons. If the test for linear trend was not significant ($p>0.05$), a Bonferroni adjustment was applied to the pairwise comparisons at that time point (*i.e.*, each comparison was tested at the 1.25% significance level). Adjusted means for each dose level were presented together with the significance level of each pairwise comparison and the test for linear trend. If applicable, the significance level of the pairwise comparisons after the Bonferroni adjustment was also provided.

Normality was assessed with a Shapiro-Wilk test and homogeneity of variance was assessed by plotting the residuals against the predicted values for the model. If significant non-normality could not be resolved by data transformation, the data were excluded as outliers. If the omission of an outlier did not affect the conclusion, results from the full data set were reported. Descriptive statistical methods were used to report other data.

HED's Chemistry and Exposure Branch (CEB) conducted a data analysis of the plasma and red blood cell cholinesterase to verify whether the statistical analyses conducted were appropriate. Additional information is provided in Appendix I to this DER.

6. **Pharmacokinetic analysis:** Individual plasma concentration profiles of acephate and methamidophos vs. actual sampling times were generated for each subject. Mean pharmacokinetic parameter estimates were determined from the individual parameter estimates for each dose level. Pharmacokinetic parameter estimates were determined with WinNonlin non-compartmental analysis model 200 (extravascular dosing; WinNonlin version 1.1, Pharsight Corp.).

Acephate $AUC_{(0-\infty)}$ data were analyzed for dose proportionality by ANOVA techniques with the male subject data only.

$$\log\big(\mathrm{AUC}(0-\infty)\big) = \mu + \beta * \log(\mathrm{Dose})$$

or

$$\mathrm{AUC}(0-\infty) = \alpha * \mathrm{Dose}\beta$$

The estimate obtained for β is a measure of dose proportionality; proportionality requires that β = 1. Dose independent parameters are implied if β = 0.

7. Method Validation and Storage/Stability

Plasma

Analytical method validation and storage stability results for plasma and study data tables for acephate and methamidophos in plasma can be found in Appendix T of the study report (MRID 45388301). Upon receipt, samples were immediately stored at -80°C until analyzed using validated Analytical Method 6696 (Iveresk Project No. 366968).

This analytical method evaluated: 1) system suitability, 2) assay specificity, 3) assay linearity, 4) assay limit of detection, 5) assay limit of quantification, 6) intraday and interday assay accuracy and precision, and 7) determination of acephate and methamidophos in human plasma from spiked human blood.

Storage stability analyses for acephate and methamidophos in human plasma when stored for a period of 8 weeks at approximately -80°C were conducted.

Urine

Analytical method validation for the determination of acephate and methamidophos in human urine, and storage stability results, can be found in Appendix U of the study report (MRID 4538801). Samples were stored at -80° C until analyzed using validated Analytical Method 6695 (Iveresk Project No. 366952).

In the method, aliquots of urine (1 mL) are spiked with acephate and methamidophos (for calibration standard and quality control samples). Samples are diluted with the addition of acetone containing polyethylene glycol (PEG). Aliquots of the diluted samples are analyzed by gas chromatography and flame photometric detection. The analytical method evaluated: 1) system suitability, 2) assay specificity, 3) assay limit of detection, 4) assay limit of quantification, 5)assay linearity, 6) intraday and interday assay accuracy and precision, and 7) determination of acephate and methamidophos in human urine samples over the concentration range of approximately 10 to 1000 ng/mL.

Storage stability analyses for acephate and methamidophos in human urine when stored for a period of 12 weeks at approximately -80°C were conducted.

II. RESULTS

A. ANALYTICAL/METHOD VALIDATION – PLASMA:

Analytical Method No. 6696 is acceptable in terms of system suitability, assay specificity, assay linearity, assay limit of detection and intraday and interday assay accuracy and precision, and therefore validated for the determination of acephate and methamidophos in human plasma in the concentration range of approximately 10-1000 ng/mL. The assay limit of quantification for both acephate and methamidophos is approximately 10 ng/mL. The analytical method validation report and storage stability results are located in Appendix T of the study report (MRID 45388301).

TABLE 2. Evaluation of gas chromatography critical parameters for assay of plasma acephate and methamidophos

Parameter	Acephate	Methamidophos
Column efficiency (# of theatrical plates)	143000	53000
Tailing factor	1.11	1.26
Precision (%)[a]	2.8 (at 25.15 ng/mL) 0.9 (at 503.0 ng/mL)	4.1 (at 25.10 ng/mL) 1.8 (at 502 ng/mL)
Plasma limit of detection (ng/mL)	6 ng/mL (3 pg on-column)	1.5 ng/mL (0.75 pg on-column)
Plasma limit of quantification (ng/mL)	10 ng/mL	10 ng/mL
Linearity of detector response (% deviation; actual amount of acephate in pg on-column)	+3.7%; 49.65 -5.9; 124.7 -5.4; 249.3 -3.9; 496.5 -2.5;1247 +7.3; 2493 +6.8; 4985	+2.2; 50.10 -2.8; 125.3 -3.7; 250.5 -5.7; 501.0 +1.8; 1253 +4.3; 2505 +3.8; 5010
Intraday and Interday Overall Accuracy (mean, %) 25.13 ng/mL 100.5 ng/mL 753.8 ng/mL	 +0.8% +0.3% +0.3%	 -5.8% -1.6% +1.5%
Overall Intraday and Interday Assay Precision (%) 25.13 ng/mL 100.5 ng/mL 753.8 ng/mL	 5.8% 7.1% 9.8%	 4.6% 5.3% 4.8%

[a] Coefficient of variation based on 10 samples
Data taken from tables beginning on page 1096 of MRID 45388301.

B. ANALYTICAL/METHOD VALIDATION – URINE:

Analytical method validation and storage stability results for the determination of acephate and methamidophos in human urine is located in Appendix U of the study report (MRID 45388301). Analytical method no. 6695 is satisfactory in terms of system suitability, assay specificity, assay linearity, assay limit of detection and intraday and interday assay accuracy and precision, and is validated for the determination of acephate and methamidophos in human urine over the concentration range of approximately 10 to 1000 ng/mL. The assay limit of quantification for both analytes was found to be approximately 10 ng/mL. Over-range human urine samples prepared around 2000 and 5000 ng/mL acephate and

methamidophos were successfully diluted with control human urine and extracted without loss of accuracy or precision.

TABLE 3. Evaluation of gas chromatography critical parameters for assay of urine acephate and methamidophos

Parameter	Acephate	Methamidophos
Column efficiency (# of theatrical plates)	160000	44900
Tailing factor	1.42	1.23
Precision (%)[a]	10.3 (at 24.93 ng/mL) 5.4 (at 498.5 ng/mL)	4.4 (at 25.05 ng/mL) 3.5 (at 501 ng/mL)
Plasma limit of detection (ng/mL)	7 ng/mL (3.5 pg on-column)	4.0 ng/mL (2.0 pg on-column)
Plasma limit of quantification (ng/mL)	10 ng/mL	10 ng/mL
Linearity of detector response (% deviation; actual amount of acephate in pg on-column)	+0.1%; 49.65 -0.2; 124.7 NC; 249.3 -0.2; 496.5 +2.4;1247 -5.8; 2493 +3.8; 4985	-0.7; 50.10 -2.5; 125.3 +11.5; 250.5 -5.3; 501.0 +1.3; 1253 -6.5; 2505 +2.3; 5010
Intraday and Interday Overall Accuracy (mean, %) 25.13 ng/mL 100.5 ng/mL 753.8 ng/mL	 -3.9% -0.4% -2.1%	 -2% -5.1% -5.6%
Overall Intraday and Interday Assay Precision (%) 25.13 ng/mL 100.5 ng/mL 753.8 ng/mL	 6.1% 8.6% 8.4%	 6.1% 7.0% 6.2%
Effect of Dilution on Accuracy and Precision (mean % deviation from actual)	-6.7% (at 2515 ng/mL) -6.3% (at 5030 ng/mL)	+1.0% (at 2510 ng/mL) +0.8 (at 5020 ng/mL)

[a] Coefficient of variation based on 10 samples
NC = not calculated

C. <u>STORAGE STABILITY IN HUMAN PLASMA</u>: Results of the storage stability study are shown in Table 4. For acephate in plasma, all analyses revealed that stability remained within 15% of the original concentration. For methamidophos in plasma: after 1 week of storage, 2 out of 5 of the replicate samples at 25 ng/mL and 1 of the 5 replicates at 750 ng/mL were outside of the ±15% of the actual concentration; after 8 weeks of storage, 1 replicate (of 5) at 100 ng/mL was outside the ±15% of the actual concentration. Overall, the mean calculated concentrations for methamidophos in plasma were within ±15% of the actual concentration and coefficients of variation (CV) were <15% at each concentration. These results indicate acceptable stability for both acephate and methamidophos in plasma when stored at approximately 80°C for a period of approximately 8 weeks.

TABLE 4. Storage stability of acephate and methamidophos in human plasma at -80°C			
Test Material (Concentration)	**Storage Duration (Weeks)**	**Mean Calculated[a] Concentration (ng/mL)**	**Mean Percent of Actual Concentration/CV (ng/mL)**
Acephate (25.13 ng/mL)	0	24.8	98.5/6.9
	1	25.7	102.1/5.6
	4	25.0	99.5/8.1
	8	25.9	103.2/3.3
Acephate (100.5 ng/mL)	0	99.5	99.0/2.6
	1	104	103.5/5.1
	4	100.7	100.2/3.1
	8	98.2	97.7/4.0
Acephate (753.8 ng/mL)	0	746	98.9/6.1
	1	746	99.0/3.3
	4	739	100.2/3.1
	8	710	94.2/2.1
Methamidophos (25.13 ng/mL)	0	27.4	109.1/3.0
	1	21.6	86.1/3.0
	4	24.8	98.1/2.4
	8	23.2	92.5/4.7
Methamidophos (100.5 ng/mL)	0	104	103.2/2.2
	1	87.5	87.1/1.4
	4	88.7	86.3/3.0
	8	86.4	85.9/1.9
Methamidophos (753.8 ng/mL)	0	750	99.6/2.6
	1	643	85.3/1.1
	4	657	87.1/1.6
	8	872	89.0/1.4

[a] Values are means of five samples
CV = coefficient of variation
Data taken from Tables 1-6 from p. 1158 - 1163 of MRID 45388301.

D. STORAGE STABILITY IN URINE: Results of the storage stability in human urine are shown in Table 5. The results indicate that acephate and methamidophos are stable in human urine when stored for a period of 12 weeks at approximately -80°C.

Acephate: At ~100 ng/mL, 3 out of 5 replicates were outside of the acceptance criteria for accuracy after 8 weeks of storage and the mean calculated concentration was outside of 100 ± 15% of the actual concentration. The CV was <15%. At ~750 ng/mL, 3 out of 5 replicates were outside of the acceptance criteria for accuracy after 8 weeks storage and the mean calculated concentration was outside of 100 ± 15% of the actual concentration. The CV was <15%. All other mean results were within 100 ± 15% of the actual concentration, including all results following 12 weeks of storage at each concentration. This indicates that the results that were outside of 15% of the actual concentration at 8 weeks do not significantly show instability.

Methamidophos: All mean results were within 100 ± 15% of the actual concentrations and the CVs for each storage period were <15%.

Overall, results indicate acceptable stability of acephate and methamidophos in human urine at concentrations of approximately 25, 100, and 750 ng/mL for a period of 12 weeks when stored at -80°C.

Table 5. Storage stability of acephate and methamidophos in human urine at -80°C

Test Material (Concentration)	Storage Duration (Weeks)	Mean Calculated[a] Concentration (ng/mL)	Mean Percent of Actual Concentration/CV (ng/mL)
Acephate (25.13 ng/mL)	0	24.2	96.4/8.8
	1	28.6	113.8/8.4
	4	23.4	93.0/12.2
	8	22.4	89.0/3.2
	12	24.7	98.2/7.7
Acephate (100.5 ng/mL)	0	99.3	98.9/2.8
	1	102.5	102.1/4.1
	4	91.8	91.4/2.1
	8	82.7	82.3/7.2
	12	100	99.5/6.3
Acephate (753.8 ng/mL)	0	742	98.4/2.1
	1	746	99.0/3.2
	4	754	100.0/5.7
	8	830	83.6/4.5
	12	718	95.0/3.5
Methamidophos (25.13 ng/mL)	0	26.1	104/3.5
	1	23.8	94.5/2.1
	4	28.1	111.9/3.0
	8	26.6	105.7/2.4
	12	25.3	100.7/3.6
Methamidophos (100.5 ng/mL)	0	94.4	94/2.9
	1	91.3	90.8/3.2
	4	89.2	88.7/2.6
	8	109	108.3/6.9
	12	97.9	97.4/3.5
Methamidophos (753.8 ng/mL)	0	701	93.0/1.7
	1	892	91.8/2.2
	4	741	98.3/1.0
	8	835	110.8/3.3
	12	740	98.2/2.5

[a] Values are means of five samples
CV = coefficient of variation
Data taken from Tables 1-6 from p. 1279-1284 of MRID 45388301.

E. DISPOSITION OF SUBJECTS: A total of 63 males and 12 females were screened for inclusion in the present study. Forty male and 10 female volunteers were selected for inclusion and all 50 subjects selected completed the study. The mean age for male subjects was 32.3 ± 7.8 years; all were white, except for a single male of Asian origin. The mean age for female subjects was 32.2 ± 7.3 years; all were white.

F. CLINICAL SIGNS: There were no adverse clinical signs that were considered related to treatment. There were no adverse clinical events in males dosed at 0.35 or 1.25 mg/kg or in the females administered the placebo. A single male in the placebo group (No. 038) had

dyspepsia at 30 minutes post-administration that was considered related to the change in diet in clinic. A single male at 0.70 mg/kg (No. 020) experienced leg pains at 30 hours post-dose that were considered related to muscle cramps. In the 1.00 mg/kg group, a single male (No. 027) reported a headache at 22 hours post-dose that was considered unrelated to treatment. In addition, the same subject reported a cough and sore throat at 9.5 and 25 hours post-dose, respectively, that were considered probably due to a viral infection. In the females administered 1.00 mg/kg, a single subject (No. 045) reported dizziness at 52 hours post-dose that was considered unrelated to treatment. No serious adverse events were reported.

G. VITAL SIGNS: There were no treatment-related effects on vital signs, ECG readings, or physical examinations during the present study.

H. CLINICAL PATHOLOGY FINDINGS: There were no treatment-related or clinically significant effects on hematology, clinical chemistry, or urinalysis parameters during the course of the study.

I. PHARMACOKINETIC ANALYSES: The concentration-profiles of acephate and methamidophos in plasma after a single oral dose of acephate at 0.35-1.25 mg/kg to male volunteers and at 1.00 mg/kg to female volunteers are presented in Table 6 and shown graphically in Figures 1-4. The mean pharmacokinetic parameter estimates derived from these concentration-time profiles are presented by compound and sex in Table 7.

No quantifiable concentrations of acephate or methamidophos were noted in samples from the placebo-treated subjects or in any pre-dose samples from any acephate treatment group. The acephate plasma concentration vs. time profiles were consistent with oral dose administration with no evidence of a significant lag phase (measurable concentrations at 1 hour post-dose at all dose levels) with mean T_{max} estimates of 1.3-2.7 hours (range: 1 to 4 hours, except for a single value of 8 hours at 0.70 mg/kg). Acephate concentrations declined rapidly up to 24 hours post-dose with mean $t_{1/2el}$ estimates of 4.4-5.4 hours (range: 3.5-6.6 hours). Mean oral clearance (CL/F) was generally slow, with mean values of 77-105 mL/hour/kg (range: 62-136 mL/hour/kg). Overall systemic exposure (AUC) demonstrated generally dose-related increases (slightly greater than dose proportional). There were no sex-related differences in pharmacokinetic parameters at 1.00 mg/kg.

Plasma concentration data were sparser for the metabolite, methamidophos, at ≤0.70 mg/kg acephate but valid C_{max}, T_{max}, and AUC_{0-t} estimates were determinable at ≥0.70 mg/kg acephate, with valid estimates for the remaining parameters at ≥1.00 mg/kg acephate. Similar to acephate, mean T_{max} estimates of 3.3-4.3 hours (range: 1 to 4 hours, except for a single value of 8 hours at 0.70 mg/kg). Methamidophos concentrations declined rapidly up to 12 hours post-dose, with mean $t_{1/2el}$ estimates of 6.7-8.3 hours (range: 3.5-11.6 hours). Mean oral clearance (CL/F) was rapid, with mean values of 3377-4439 mL/hour/kg (range: 2503-7800 mL/hour/kg). Overall systemic exposure (AUC) demonstrated generally less than dose-proportional increases. There were no sex-related differences in pharmacokinetic parameters at 1.00 mg/kg.

Mean plasma concentration data for acephate and methamidophos were also outside of the method validated concentration range of 10-1000 ng/mL at several dose levels and timepoints. For acephate, mean plasma concentrations were above 1000 ng/mL at 1.0 and 1.25 mg/kg in males at 1, 2, and 4 hours post-dose, and at 1.0 mg/kg in females at 2 and 4 hours post-dose. For methamidophos, mean plasma concentrations were below 10 ng/mL at 0.70 mg/kg (2, 8, and 12 hours post-dose), 1.0 mg/kg (1 and 12 hours post-dose), and 1.25 mg/kg (1 and 12 hours post-dose) in males, and at 1.0 mg/kg (1 and 12 hours post-dose) in females.

TABLE 6. Mean (± SD) plasma concentration-time profiles (ng/mL) of acephate and methamidophos after a single oral administration of acephate to human volunteers. [a]

Time (hours)	Concentration (ng/mL)				
	Male (mg/kg)				Female (mg/kg)
	0.35	0.70	1.00	1.25	1.00
Acephate					
Pre-dose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	482.9 ± 122.1	641.3 ± 275.7	1043.3 ± 584.1	1014.9 ± 444.8	792.7 ± 714.3
2	434.0 ± 47.5	842.3 ± 340.3	1320.0 ± 179.2	1598.6 ± 240.9	1205.9 ± 406.3
4	309.7 ± 29.6	744.0 ± 208.2	1087.4 ± 158.8	1441.4 ± 220.0	1309.3 ± 243.5
8	151.1 ± 17.4	433.0 ± 104.7	658.1± 68.6	858.7 ± 134.6	701.1 ± 162.0
12	73.0 ± 12.8	235.1 ± 87.1	363.3 ± 47.4	462.4 ± 94.5	382.7 ± 120.8
24	12.1 ± 8.5	51.4 ± 17.6	83.4 ± 24.3	99.9 ± 29.4	75.0 ± 38.7
48	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
72	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
168 (Day 7)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
336 (Day 14)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Methamidophos					
Pre-dose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	0.0 ± 0.0	0.0 ± 0.0	5.64 ± 7.28	3.99 ± 6.81	4.63 ± 7.95
2	0.0 ± 0.0	8.06 ± 5.57	13.74 ± 1.78	17.81 ± 3.56	11.23 ± 8.52
4	0.0 ± 0.0	10.79 ± 5.17	19.21 ± 3.33	24.91 ± 2.99	21.76 ± 3.14
8	0.0 ± 0.0	3.57 ± 6.10	14.87 ± 3.54	19.14 ± 3.93	14.86 ± 6.87
12	0.0 ± 0.0	1.63 ± 4.31	4.89 ± 6.10	8.64 ± 6.29	7.19 ± 6.80
24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
48	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
72	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
168 (Day 7)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
336 (Day 14)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

a Data were obtained from Table Q1.2 on pages 967-971 of MRID 45388301; n = 7 sample/time point.

TABLE 7. Mean (± SD) pharmacokinetic parameter estimates derived from the plasma concentration-time profiles (ng/mL) after a single oral administration of acephate to human volunteers. [a]

Time (hour)	Concentration (ng/mL)				
	Male (mg/kg)				Female (mg/kg)
	0.35	0.70	1.00	1.25	1.00
Acephate					
C_{max} (ng/mL)	506.3 ± 86.6	921.9 ± 156.8	1451.4 ± 206.0	1688.6 ± 262.3	1515.7 ± 319.6
T_{max} (hour)	1.29 ± 0.49	2.71 ± 2.36	2.00 ± 1.00	2.43 ± 1.13	2.71 ± 1.25
$t_{1/2el}$ (hour)	4.39 ± 0.65	5.00 ± 0.34	5.42 ± 0.72	5.21 ± 0.52	4.83 ± 0.68
AUC_{0-t} (hour × ng/mL)	3277.6 ± 514.1	8063.3 ± 954.0	12,327.8 ± 667.4	15,473.9 ± 2143.6	12,847.3 ± 2288.1
$AUC_{0-\infty}$ (hour × ng/mL)	3407.1 ± 507.5	8439.4 ± 1050.2	13,000.0 ± 812.8	16,243.2 ± 2398.5	13,400.9 ± 2595.8
CL/F (mL/hour/kg)	105.0 ± 17.5	84.0 ± 10.5	77.3 ± 5.10	78.6 ± 12.9	77.0 ± 15.7
Methamidophos [b]					
C_{max} (ng/mL)	0.0 ± 0.0	12.7 ± 1.98	19.1 ± 3.50	25.2 ± 2.74	22.0 ± 2.65
T_{max} (hour)	0.0 ± 0.0	4.29 ± 1.80	3.29 ± 1.25	3.71 ± 0.76	3.57 ± 1.14
$t_{1/2}$ el (hour)	0.0 ± 0.0	8.30 ± 4.63 [c]	6.66 ± 2.21	6.92 ± 2.30	6.65 ± 2.77
AUC_{0-t} (hour × ng/mL)	0.0 ± 0.0	84.0 ± 59.2	204.8 ± 79.2	281.3 ± 76.6	236.2 ± 87.3
$AUC_{0-\infty}$ (hour × ng/mL)	0.0 ± 0.0	222.2 ± 81.3 [c]	252.8 ± 94.3	331.2 ± 89.6	284.1 ± 104.4
CL/F (mL/hour/kg)	0.0 ± 0.0	3376.5 ±1235.3 [c]	4438.9 ± 1513.6	4090.1 ± 1375.2	4110.6 ± 1951.1

a Data were obtained from Tables Q1.1.1-1.1.6 on pages 961-966 and Q2.1 on pages 972-976 of MRID 45388301; n = 7 sample/time point, unless noted otherwise.

b Mean ± SD pharmacokinetic parameter estimates for methamidophos were calculated from the individual data by the Reviewers.

c n = 2.

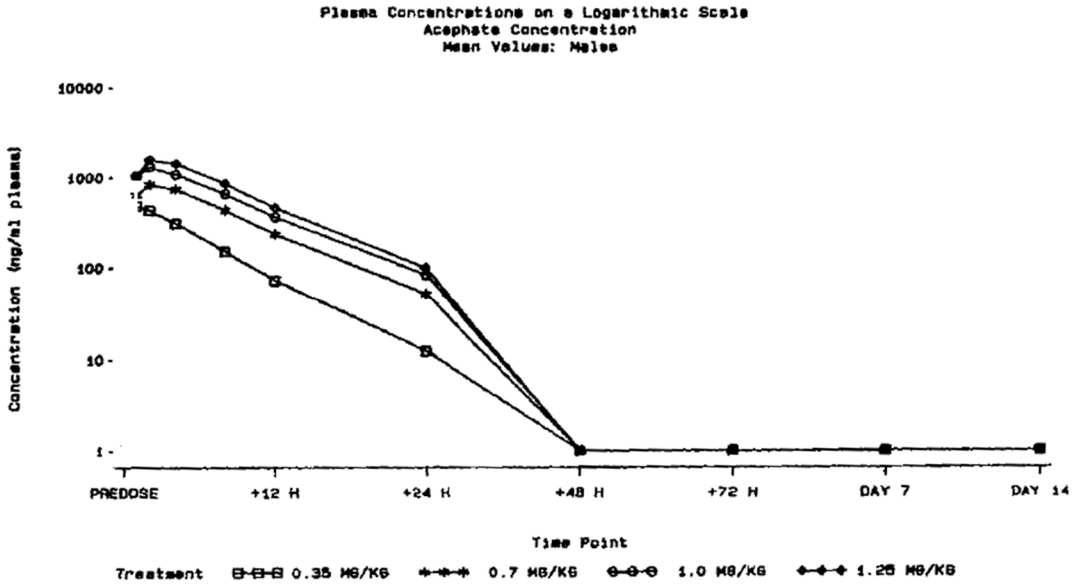


FIGURE 1. Plasma concentration-time profiles (ng/mL) of acephate after a single oral administration of acephate to human male volunteers. Figure obtained from page 879 (MRID 45388301).

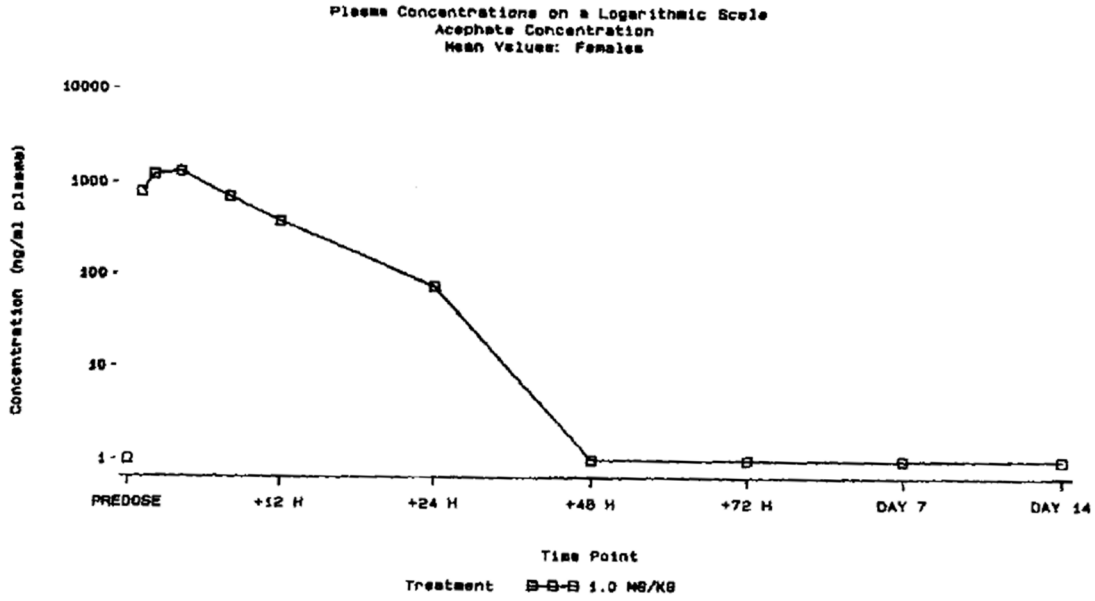


FIGURE 2. Plasma concentration-time profiles (ng/mL) of acephate after a single oral administration of acephate to human female volunteers. Figure obtained from page 881 (MRID 45388301).

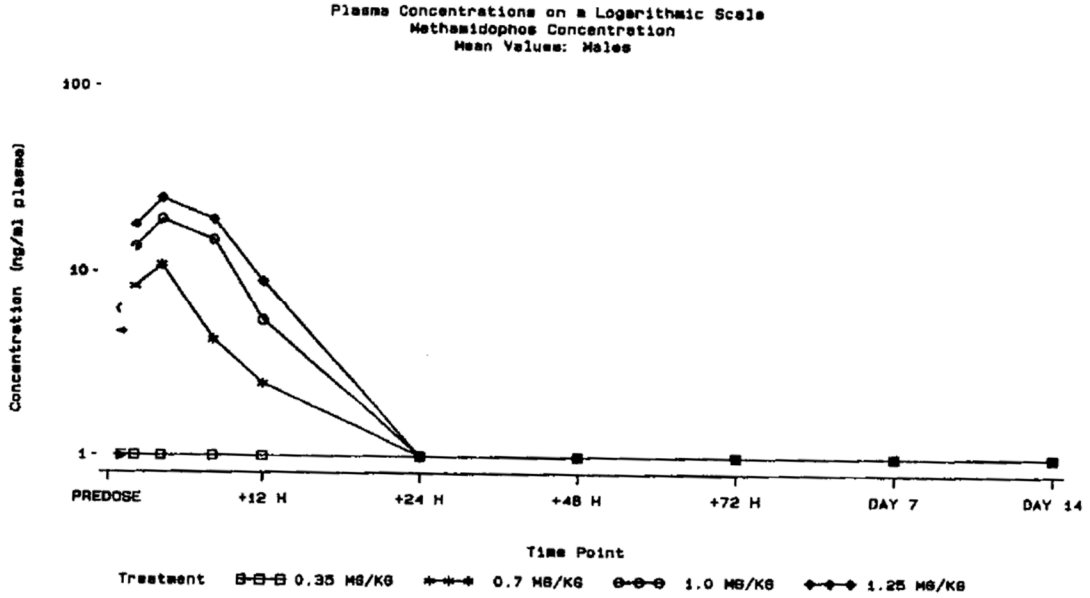


FIGURE 3. Plasma concentration-time profiles (ng/mL) of methamidophos after a single oral administration of acephate to human male volunteers. Figure obtained from page 883 (MRID 45388301).

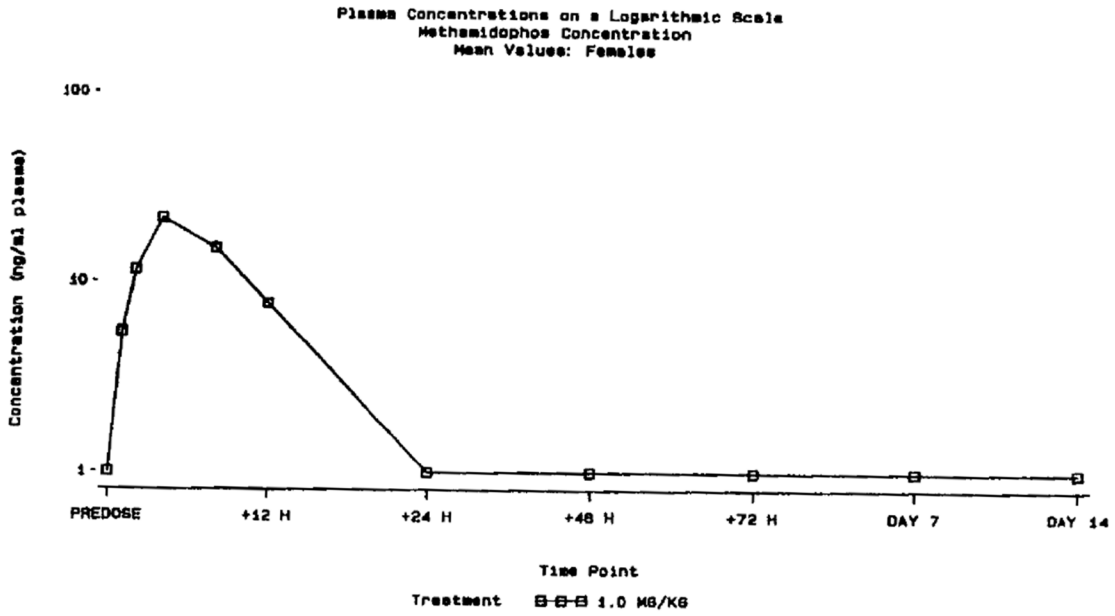


FIGURE 4. Plasma concentration-time profiles (ng/mL) of methamidophos after a single oral administration of acephate to human female volunteers. Figure obtained from page 885 (MRID 45388301).

J. URINE CONCENTRATION-TIME PROFILES: The concentration-profiles of acephate and methamidophos in urine after a single oral dose of acephate at 0.35-1.25 mg/kg to male volunteers and at 1.00 mg/kg to female volunteers, and the percentage recovery estimates of the administered dose of acephate (as acephate and methamidophos) are presented in Table 8.

The total recovery of acephate and methamidophos (as acephate equivalents) in urine through 48 hours post-dose ranged from 25.0% to 61.1% in males and 12.1% to 50.6% in females with most of the recovery occurring within the initial 12-hour post-dose period. The mean acephate recovered at 1.0 mg/kg for males was 51.7%, versus 25.7% for females. The fractions of the administered acephate dose or the total recovered test material (acephate and methamidophos [as acephate-equivalents]) recovered as methamidophos ranged from 0.16-0.91% administered dose (AD). The fate of the unrecovered administered acephate doses is unknown but might be due to incomplete absorption or additional metabolism. The lower recoveries in females vs. males was attributed to decreases in urine collection volumes and fractions of unchanged parent observed in females compared to males.

Urine concentrations of methamidophos (systemic exposure) generally decreased in parallel to acephate at ≥1.00 mg/kg acephate during the 12-48 hour collection period. Accumulation of methamidophos would not be expected due to this similarity in systemic clearance/elimination.

Mean urine concentration data for acephate and methamidophos were outside of the method validated concentration range of 10-1000 ng/mL at several dose levels/timepoints, including exceedances of 1000 ng/mL for acephate at 0-12 hours (all dose levels, both sexes), 12-24 hours (all dose levels, both sexes), and slightly above the range at 24-48 hours (1.00 and

1.25 dose levels in males). For methamidophos, concentrations were below the limit of quantification of 10 ng/mL at 24-48 hours for the 0.70 mg/kg (males) and slightly below at 1.0 mg/kg (females).

TABLE 8. Mean (± SD) urine concentration-time profiles (ng/mL) of acephate and methamidophos after a single oral administration of acephate to human volunteers. [a]

Time (hour)	Concentration (ng/mL)				
	Male (mg/kg)				Female (mg/kg)
	0.35	0.70	1.00	1.25	1.00
Acephate					
Pre-dose (−12-0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0-12	9535.7 ± 3106.5	21,457.1 ± 12,422.4	34,957.1 ± 21,973.3	27,371.4 ± 8151.6	31,000.0 ± 16,744.2
12-24	1488.1 ± 679.7	5344.3 ± 2549.9	9694.3 ± 4541.4	9328.6 ± 2113.6	7967.1 ± 4777.1
24-48	105.1 ± 43.9	545.7 ± 193.3	1249.3 ± 643.8	1020.7 ± 206.8	688.0 ± 388.8
Acephate recovered (% AD; minimum/maximum) [b]	25.0/61.1	27.6/59.0	35.5/60.6	35.3/52.5	12.1/50.6
Acephate recovered (% AD; mean ± SD)	42.8 ± 12.8	49.6 ± 10.3	51.7 ± 9.3	43.4 ± 5.3	25.7 ± 14.0
Methamidophos					
Pre-dose (−12-0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0-12	82.7 ± 24.4	187.0 ± 91.7	295.7 ± 141.3	324.6 ± 71.0	283.3 ± 133.4
12-24	22.5 ± 8.58	78.2 ± 41.7	148.7 ± 55.9	169.7 ± 38.6	118.5 ± 73.3
24-48	0.0 ± 0.0	4.73 ± 5.90	15.3 ± 9.26	17.8 ± 2.90	7.30 ± 7.19
Methamidophos recovered (% AD of acephate; minimum/maximum) [b]	0.32/0.70	0.32/0.83	0.50/0.91	0.61/0.90	0.16/0.69
Methamidophos recovered (%AD of acephate; mean ± SD)	0.52 ± 0.15	0.67 ± 0.18	0.68 ± 0.15	0.75 ± 0.12	0.34 ± 0.19
Total recovery (% AD; minimum/maximum) [b]	25.4/61.8	27.9/59.8	36.0/61.3	35.9/53.3	12.2/51.3

a Data were obtained from Table Q3.1 on pages 995-996 of MRID 45388301; n = 7 sample/time point.
b Calculated by the Reviewers from the individual data (see Appendix II).
AD Administered dose.

K. <u>PLASMA AND RBC CHOLINESTERASE</u>: The activities of plasma and RBC ChE after a single oral dose of acephate at 0.0-1.25 mg/kg to male volunteers and at 0.0 or 1.00 mg/kg to female volunteers are presented in Table 9 and shown graphically in Figures 5-8.

Plasma ChE activities were decreased ($p<0.01$) by 9-13% at 12, 24, 48, and 72 hours post-dose in the 1.25 mg/kg males with significant ($p<0.01$) linear trend tests at 48 and 72 hours post-dose. Plasma ChE activities were decreased ($p<0.05$) by 10-13% at 8, 12 and 24 hours post-dose in the 1.00 mg/kg females. There were no treatment-related effects on plasma ChE activities at ≤1.00 mg/kg in the male subjects. Decreases in plasma ChE activities in males at 0.35 mg/kg at 12 and 72 hours post-dose demonstrated no relationship to dose or time. For RBC ChE activities, decreases ($p<0.01$) of 7% and 15% were noted in the 1.25 mg/kg males and 1.00 mg/kg females, respectively, at 12 hours post-dose. Increases ($p<0.05$) of 8-9% at 48 and 72 hours post-dose in the 0.35 mg/kg males were considered unrelated to dose; there were no other treatment-related effects on RBC ChE activities at ≤1.00 mg/kg in the male subjects.

In male subjects, concurrent decreases ($p<0.01$) in both plasma and RBC ChE activities were noted only at 12 hours post-dose in the 1.25 mg/kg group, with decreases of 13% and 7%, respectively. The further decreases ($p<0.01$) in plasma ChE activities at 24, 48, and 72 hours in the 1.25 mg/kg males were considered not relevant toxicologically as they were <10%, occurred concomitantly with low or non-detectable plasma concentrations of acephate, and occurred without concomitant effects on RBC ChE concentrations. In female subjects, the concurrent decreases ($p<0.05$) in both plasma and RBC ChE activities were noted only at 12 hours post-dose at 1.00 mg/kg, with decreases of 12% and 15%, respectively. The further decrease ($p<0.001$) in plasma ChE activity at 24 hours was considered not relevant toxicologically as it occurred concomitantly with a decreasing plasma concentration of acephate and occurred without concomitant effects on RBC ChE activity. Additionally, RBC ChE measurements are generally preferred over plasma measures of ChE because data on RBCs may provide a better representation of the inhibition of the neural target enzyme, AChE[2].

There was variability in the data at baseline and within the placebo groups. CVs for plasma ChE at baseline were 28%, 16%, 23%, and 11% at 0.35, 0.70, 1.0, and 1.25 mg/kg in males, respectively. The CV for plasma ChE at baseline in the 1.0 mg/kg females was 21%. Changes and statistical significance for each dose group were compared to baseline values at each dose level (not to placebo group controls). In most instances (except for the 1.25 mg/kg males), all changes observed were within the CV of baseline values for plasma ChE. In the placebo groups, CVs at baseline were approximately 13% in males, and 14% in females. Therefore, all changes in plasma ChE were within the variability of baseline values at each dose level (with the exception of the 13% change at 12-hours in the 1.25 mg/kg males), as well as within the variability of placebo group controls. The difference between the baseline value for the placebo group and the baseline value for the 1.25 mg/kg males was 8%.

There was less variability in the results for RBC ChE, however, results were still within variability of baseline values for males, and slightly outside for females (11% change at 1.0 mg/kg compared to baseline CV of 9%). Changes observed in RBC ChE in males were also mostly within the variability of placebo group baseline of 8% (there was a 9% increase in RBC ChE at 72 hours in the low dose males, but increases aren't toxicologically relevant). The placebo baseline in females had a CV of 4%, so the 11% change in RBC ChE in females at 12 hours, which was also statistically significant, was outside of the CV of baseline for that dose group, as well as the placebo baseline.

[2] "Office of Pesticide Programs Science Policy on The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides". OPP, USEPA, 2000. https://www.epa.gov/sites/default/files/2015-07/documents/cholin.pdf.

TABLE 9. Mean (± SD) plasma and RBC ChE concentrations (IU/L) after a single oral administration of acephate to human volunteers. [a]

Time (hour)	Male (mg/kg)					Female (mg/kg)	
	0.0 [b]	0.35	0.70	1.00	1.25	0.0 [c]	1.00
Plasma ChE							
Baseline	*5440.3 ± 690.7*	*5525.4 ± 1526.9* (CV=28%)	*5151.9 ± 824.5* (CV=16%)	*5776.3 ± 1332.5* (CV=23%)	*5002.7 ± 565.6* (CV=11%)	*4310.7 ± 608.3*	*4702.4 ± 979.3* (CV=21%)
1	5038.3 ± 703.2	5078.7 ± 1588.3	4631.3 ± 935.0 (↓10)	5450.9 ± 1253.7	4691.9 ± 569.7	4116.7 ± 617.2	4276.0 ± 907.8
2	5152.5 ± 712.7	5145.4 ± 1505.1	4664.9 ± 962.8	5505.3 ± 1258.6	4774.4 ± 660.7	4182.3 ± 754.7	4338.3 ± 934.7
4	5085.8 ± 670.7	5186.7 ± 1616.9	4804.9 ± 824.9	5515.1 ± 1400.6	4683.7 ± 567.0	4290.3 ± 679.0	4406.9 ± 981.9
8	5070.6 ± 629.7	5028.7 ± 1458.0	4743.7 ± 930.8	5500.4 ± 1255.2	4459.1 ± 568.7	4286.7 ± 1140.6	4111.1 ± 938.3*[e] (↓13)
12	5160.4 ± 630.6	4942.6 ± 1430.4* (↓11)[d]	4641.6 ± 881.7 (↓10)	5613.7 ± 1351.9	4365.1 ± 563.4** (↓13)	4211.3 ± 671.5	4141.0 ± 930.7* (↓12)
24	5371.5 ± 800.9	5239.7 ± 1613.2	4934.7 ± 1002.1	5695.0 ± 1361.5	4550.4 ± 475.8** (↓9)	4467.3 ± 741.1	4222.9 ± 1004.7*** (↓10)
48	5403.7 ± 621.5	5597.4 ± 1596.5	4900.3 ± 757.1	5548.6 ± 1337.5	4544.3 ± 502.5*** (↓9)	4604.0 ± 749.7	4716.1 ± 1048.3
72	5494.4 ± 618.4	5296.7 ± 1353.0 (↓4)	5106.3 ± 744.2	5765.7 ± 1326.5	4579.1 ± 646.0*** (↓9)	4363.3 ± 714.0	4529.0 ± 1036.5
168 (Day 7)	5297.8 ± 691.8	5336.9 ± 1462.6	5191.1 ± 685.7	5531.3 ± 1295.4	4758.4 ± 490.0	3986.7 ± 562.6	4388.4 ± 1037.7
336 (Day 14)	5303.8 ± 736.8	5270.4 ± 1544.8	5169.0 ± 789.3	5892.7 ± 1503.6	4987.3 ± 440.2	4287.7 ± 1022.4	4961.6 ± 1598.0
RBC ChE							
Baseline	*12493.0 ± 1176.1*	*12026.7 ± 1156.6* (CV=10%)	*11647.0 ± 775.9*	*12389.7 ± 728.6*	*12014.4 ± 964.3* (CV=8%)	*12072.0 ± 464.1*	*12099.4 ± 1075.6* (CV=9%)
1	12419.4 ± 1359.2	12363.7 ± 1486.5	11616.4 ± 1245.3	12686.6 ± 1112.4	12371.7 ± 1097.3	11011.3 ± 397.8	11549.0 ± 1191.8
2	12902.3 ± 1396.4	12472.7 ± 1378.8	11881.4 ± 1240.5	12460.0 ± 929.5	12293.1 ± 1026.4	11583.0 ± 686.6	11828.3 ± 1106.4
4	12725.6 ± 1042.2	12822.3 ± 1655.7	11413.1 ± 1182.6	12914.1 ± 1340.7	12056.3 ± 1212.3	12090.0 ± 211.5	11562.4 ± 1077.8
8	12670.3 ± 1540.9	11936.9 ± 1509.7	11658.7 ± 940.7	12439.6 ± 1274.7	11089.4 ± 788.0	11790.3 ± 867.7	12052.4 ± 1760.2
12	12793.2 ± 1498.9	12116.7 ± 1333.7	12603.0 ± 1637.9	12099.7 ± 1536.9	11189.3 ± 1432.7** (↓7)	11875.7 ± 356.6	10746.1 ± 1247.4** (↓11)
24	12535.8 ± 1397.5	12337.7 ± 1439.9	11437.9 ± 922.9	11590.4 ± 985.2	11644.6 ± 989.5	11055.0 ± 577.7	11483.1 ± 829.2
48	12493.2 ± 1303.4	12978.3 ± 1556.6* (↑8)	11235.9 ± 864.4	12193.0 ± 1630.0	11624.9 ± 826.5	11829.3 ± 119.3	11427.4 ± 1125.3
72	12100.9 ± 1478.8	13026.0 ± 1159.6*** (↑9)	10603.9 ± 686.7	11768.3 ± 2140.3	11887.7 ± 1043.3	10409.0 ± 1087.4	10647.0 ± 1386.6 (↓12)
168 (Day 7)	12325.4 ± 937.4	12079.7 ± 1561.5	12263.3 ± 1114.0	12561.9 ± 1095.8	11457.6 ± 1273.0	10843.0 ± 572.8	10796.6 ± 1158.5
336 (Day 14)	12573.3 ± 1327.7	12399.1 ± 1574.2	11511.3 ± 819.4	11969.4 ± 1976.4	12636.4 ± 1017.4	12139.0 ± 625.3	12133.6 ± 1344.9

a Data were obtained from Tables 5, 6, 7, and 8 on pages 74, 75, 77, and 79 and Tables M2.1 and M2.2 on pages 703-716 of MRID 45388301; n = 7 sample/time point, unless noted otherwise.
b n = 12.
c n = 3.
d Percent changes and statistical analysis are calculated from baseline.
e Statistically significant when outliers were not excluded.
* Significantly different from control (baseline for each dose group): p<0.05 (*); p<0.01 (**); p<001 (***).
CV = coefficient of variation.

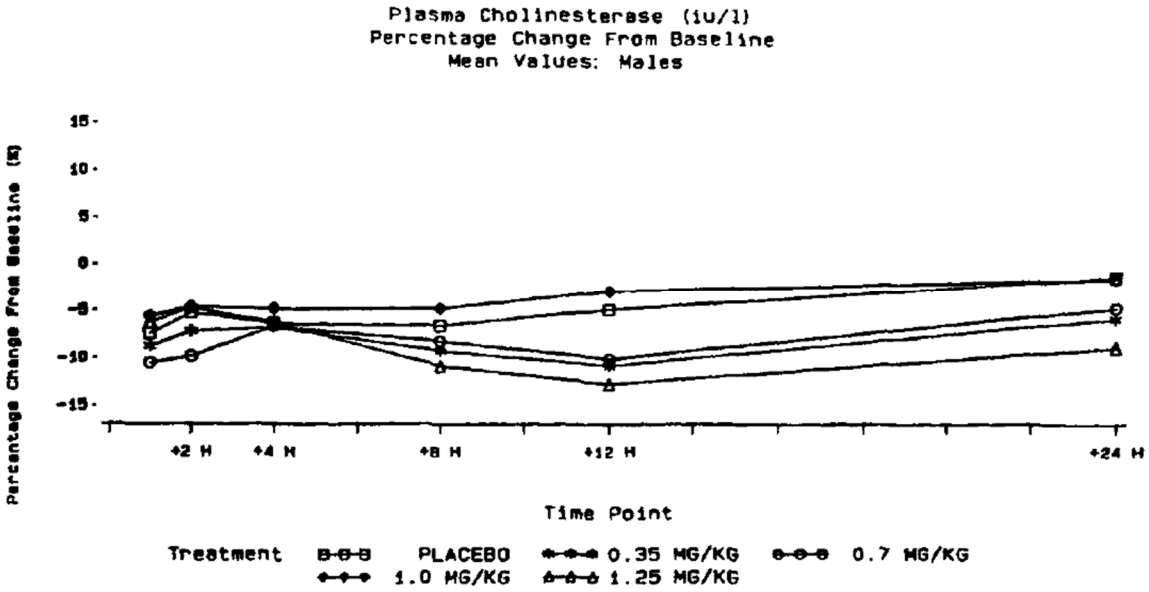


FIGURE 5. Plasma cholinesterase concentration-time profiles (IU/L) after a single oral administration of acephate to human male volunteers.

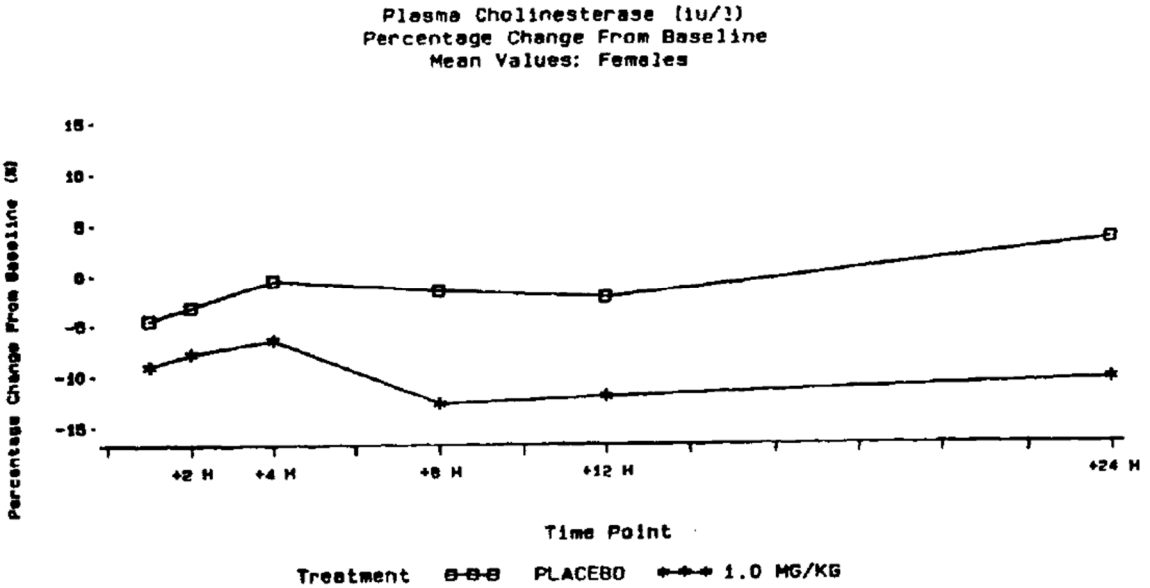


FIGURE 6. Plasma cholinesterase concentration-time profiles (IU/L) after a single oral administration of acephate to human female volunteers.

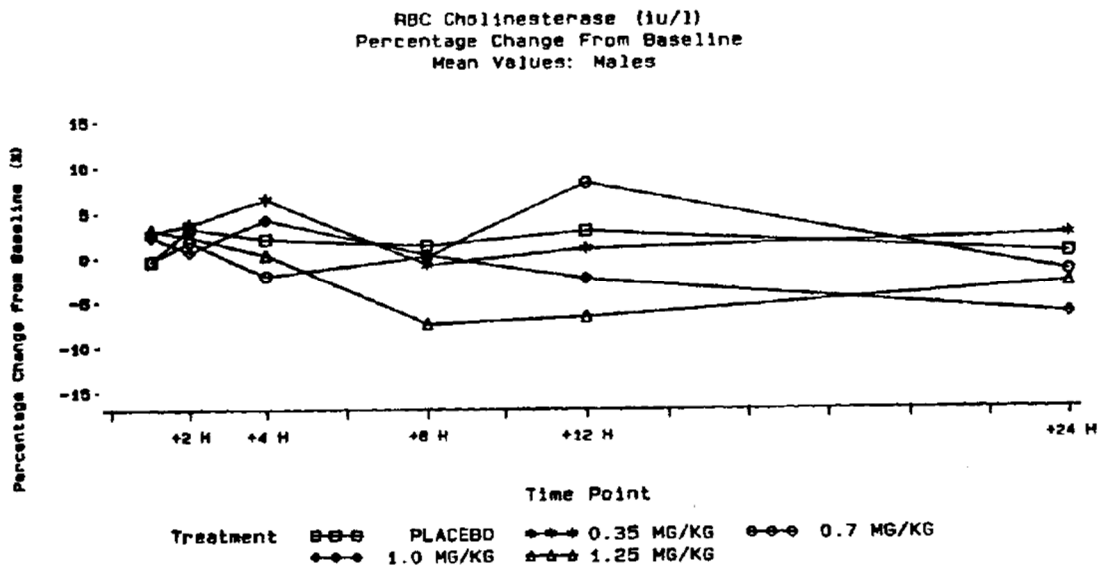


FIGURE 7. Red blood cell cholinesterase concentration-time profiles (IU/L) after a single oral administration of acephate to human male volunteers.

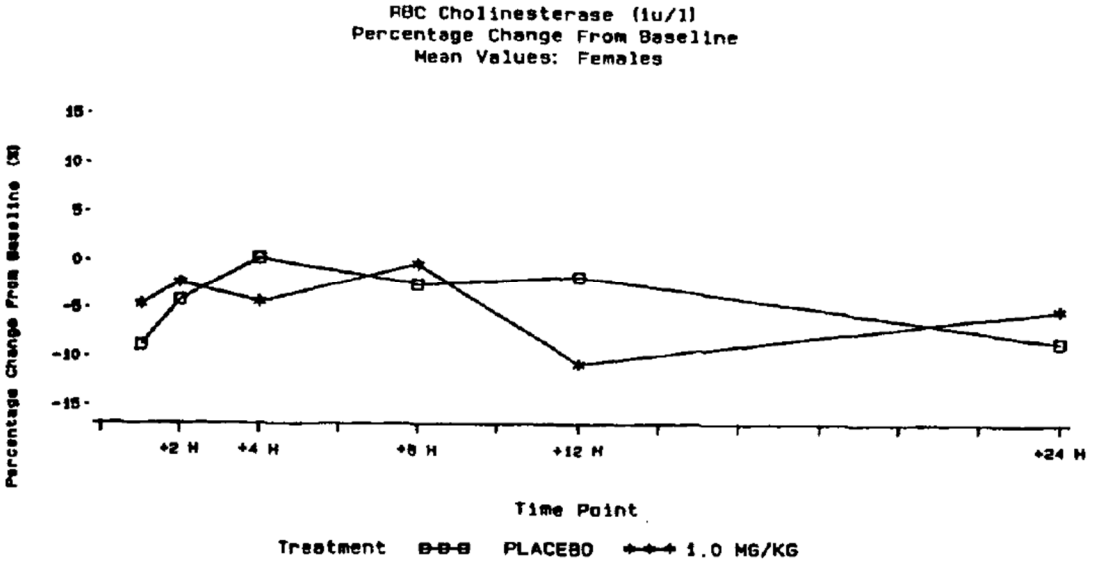


FIGURE 8. Red blood cell cholinesterase concentration-time profiles (IU/L) after a single oral administration of acephate to human female volunteers.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: There were no observed effects of acephate technical administration noted in any subject and no clinically-relevant decreases in plasma or RBC ChE activities were observed. Therefore, the NOEL for single-dose oral administration of acephate to human volunteers was 1.25 mg/kg in males and 1.00 mg/kg in females.

B. REVIEWER COMMENTS: There were no treatment-related effects on clinical signs, vital signs, or clinical pathology findings.

The acephate plasma concentration vs. time profiles were consistent with oral dose administration with no evidence of a significant lag phase. Mean T_{max} estimates ranged from 1.3-2.7 hours. Acephate concentrations declined rapidly up to 24 hours post-dose post-C_{max}, with mean $t_{1/2el}$ estimates of 4.4-5.4 hours. Mean CL/F estimates were generally slow, with values of 77-105 mL/hour/kg. Overall systemic exposure demonstrated generally dose-related increases (slightly greater than dose proportional). There were no sex-related differences in pharmacokinetic parameters at 1.00 mg/kg (the single dose level in female subjects).

Plasma concentration data were sparser for the metabolite, methamidophos, but valid C_{max}, T_{max}, and AUC_{0-t} estimates were determinable at ≥0.70 mg/kg acephate, with valid estimates for the remaining parameters at ≥1.00 mg/kg acephate. Mean T_{max} estimates ranged from 3.3-4.3 hours. Methamidophos concentrations declined rapidly up to 12 hours post-dose post-C_{max}, with mean $t_{1/2el}$ estimates of 6.7-8.3 hours. Mean CL/F estimates were rapid, with values of 3377-4439 mL/hour/kg. Overall systemic exposure demonstrated generally less than dose-proportional increases. There were no sex-related differences in pharmacokinetic parameters at 1.00 mg/kg.

The total recovery of acephate and methamidophos (as acephate equivalents) in urine through 48 hour post-dose ranged from 25% to 62% in males and 12% to 51% in females, with most of the recovery occurring within the initial 12-hour post-dose period. The fractions of the administered acephate dose recovered as methamidophos were <1% AD and ≤2% of the total recovered dose and were independent of dose or sex. Urine concentrations of methamidophos (systemic exposure) generally decreased in parallel to acephate at ≥1.00 mg/kg acephate and accumulation of methamidophos would not be expected.

Plasma ChE activities were decreased ($p<0.01$) by 9-13% at 12, 24, 48, and 72 hours post-dose in the 1.25 mg/kg males with significant ($p<0.01$) linear trend tests at 48 and 72 hours post-dose. Plasma ChE activities were decreased ($p<0.05$) by 10-13% at 8, 12 and 24 hours post-dose in females (1.00 mg/kg). For RBC ChE activities, decreases ($p<0.01$) of 7% and 11% were noted in the 1.25 mg/kg males and the 1.00 mg/kg females, respectively, at 12 hours post-dose. In male subjects, decreases ($p<0.01$) in both plasma and RBC ChE activities were noted only at 12 hours post-dose in the 1.25 mg/kg group. In female subjects, the decreases ($p<0.05$) in both plasma and RBC ChE activities were noted only at 12 hours post-dose.

Analytical methods and storage/stability for the determination of acephate and methamidophos in human blood and urine were described and validated. The analytical methods (6696 for human blood/plasma, and 6695 for human urine), were validated for the determination of acephate and methamidophos at a concentration range of approximately 10 to 1,000 ng/mL.

There were several results that fell outside of the method validated range of 10 to 1,000 ng/mL for both human blood/plasma and urine. Mean urine concentration data for acephate and methamidophos were outside of the method validated concentration range at the with exceedances of 1,000 ng/mL for acephate at 0-12 hours (all dose levels, both sexes), 12-24 hours (all dose levels, both sexes), and 24-48 hours (1.00 and 1.25 dose levels (males). For methamidophos, concentrations were below the limit of quantification of 10 ng/mL at 24-48 hours for the 0.70 mg/kg (males) and 1.0 mg/kg (females). Mean plasma concentration data for acephate and methamidophos were also outside of the method validated concentration range of 10-1000 ng/mL at several dose levels and timepoints. For acephate, mean plasma concentrations were above 1000 ng/mL at 1.0 and 1.25 mg/kg in males at 1, 2, and 4 hours post-dose, and at 1.0 mg/kg in females at 2 and 4 hours post-dose. For methamidophos, mean plasma concentrations were below 10 ng/mL at 0.70 mg/kg (2, 8, and 12 hours post-dose), 1.0 mg/kg (1 and 12 hours post-dose), and 1.25 mg/kg (1 and 12 hours post-dose) in males, and at 1.0 mg/kg (1 and 12 hours post-dose) in females.

This study is classified **acceptable/non-guideline**.

The following deficiencies were identified:

- Several values for both acephate and methamidophos in urine and blood/plasma were outside of the method validated range of 10-1,000 ng/mL. However, information from the study is still considered useful for improving predictability of future PBPK modeling.

Appendix I

Statistical Summary

Data analyses of plasma cholinesterase (ChE) and red blood cell (RBC) ChE measurements in this study were reviewed by CEB/HED and summarized below (Nguyen, J., D466156, 10-FEB-2023; TXR# 0058543).

The registrant used "a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (i.e., 1, 2, 4, 8, 12, 24, 48, 72 hours, day 7, and day 14) and dose level by timepoint interaction" to analyze the percent change from baseline of plasma ChE and RBC ChE, where each individual subject's plasma ChE (or RBC ChE) baseline value was the average of measurements before dosing. Both a linear trend test of dose response and then pairwise comparisons of dose groups vs. the placebo were performed at each timepoint to determine the timepoint at which statistically significant increasing dose-response trends were observed and/or statistically significant different responses between dose groups and the placebo group were observed. The result of the linear trend test was used to determine whether the pairwise comparisons of the dose groups vs. placebo at each timepoint were adjusted for multiple comparisons. More specifically, if the p-value of the linear trend test at a specific timepoint was > 0.05 indicating that observed dose-response trend was not statistically significant, then the registrant used the Bonferroni method to adjust for multiple comparisons when performing the pairwise comparisons at that timepoint; otherwise, if p-value of the linear trend test at a timepoint was ≤ 0.05, no adjustment was applied to the p-values of multiple pairwise comparisons performed at that timepoint.

Reviewer's comments: While the registrant indicated "a repeated measures analysis of variance (ANOVA)" was used to analyze the data, it was unclear what variance-covariance matrices the registrant considered, or which were selected for the models. Also, CEB believes the Dunnett test would be a more appropriate method to adjust for multiple comparisons of dose groups vs. the placebo group than the Bonferroni method used by the registrant, because the Bonferroni method severely sacrifices statistical power to maintain the family-wise error rate.

While reviewing the data, CEB statisticians also discovered that four subjects had extra measurements of RBC ChE (subject "028" at 72 hours, subject "036" at 48 hours, subject "038" at 72 hours, and subject "043" at 8 hours). In response to an EPA query regarding the rationale surrounding these extra measurements, the registrant stated in a responding email that the repeat measurements were taken when initial RBC ChE measurements were >20% from their baseline value. However, there were several other measurements that met this criterion and did not have repeat measurements taken. The initial measurements from the four subjects with repeat measurements were also within the "normal" ranges provided in the study report for 18-50 year old humans (page 152 of study report, MRID 45388301).

Given the unclear variance-covariance matrices being considered and selected by the registrant, the use of the Bonferroni adjustment method, and the unclear reasons for including some replicate measurements, CEB reanalyzed the plasma and RBC ChE data. CEB statisticians used mixed effects models to analyze the percent change from the baseline of plasma and RBC ChE

data. Dose group, timepoint, and the interaction between dose group and timepoint were included by CEB in the models as fixed effects. In the models, each subject was set as an experimental subject in the study design (i.e., random effect). For each specific plasma ChE or RBC ChE data of each gender, CEB explored the following variance-covariance matrices: compound symmetric (CS), CS by dose group, unstructured (UN), spatial linear (SP(LIN)), spatial power ((SP(POW)), and SP(POW) by dose group with the intent of selecting that for which the lowest Bayesian Information Criterion (BIC) value was found. Note that an autoregressive lag-1(AR(1)) variance-covariance was not considered because the time intervals between the consecutive repeated measurements were not consistent (i.e., unequal time intervals between consecutive timepoints). A linear trend test at each timepoint was implemented through an "ESTIMATE" statement in SAS using appropriate linear coefficients. Dunnett test (not Bonferroni) was used to compare dose groups to the placebo group at each timepoint.

Given the confusing and inconsistent rationale regarding the extra RBC ChE measurements of 4 subjects, CEB statisticians conducted two separate analyses for the percent change from baseline of RBC ChE as a sensitivity analysis: (i) one analysis that used the initial (first measured) replicate values and (ii) an alternative analysis that used the second replicate values. CEB elected to specifically NOT use the averages of the two replicates in the analysis since doing so would result in analyses that would be biased. This is because the four subjects and the timepoints were not randomly selected for obtaining second measurements, rather additional measurements were taken depending on the value of the initial measurement. Within each data analysis, there were a few outliers (studentized residuals > 3); given the small number of outliers, CEB statisticians only performed analyses that included the outliers. All comparisons were done with two-sided test at a significance level $\alpha = 0.05$. CEB analysis was done using SAS 9.4.

A spatial power (SP(POW)) variance-covariance matrix was selected for the male plasma ChE data, and a compound symmetric (CS) variance-covariance matrix was selected for the male RBC ChE data based on BIC values. For female plasma ChE and RBC ChE, a spatial power (SP(POW)) variance-covariance by dose group was selected for all datasets.

Overall statistical conclusions based on CEB's analyses (based on initial values only):

- ***Male Plasma ChE:*** there was evidence of acephate effect on blood plasma ChE in the 1.25 mg/kg (the percent change from baseline was significantly different from the placebo at 12, 24, 48, and 72 hours; point estimates of reduction from baseline plasma ChE were >10% at 12 hours and close to 10% at 24, 48 and 72 hours). Because there were no significant differences in the percent change from baseline plasma ChE between the dose groups 0.7 mg/kg or 1.0 mg/kg and the placebo group, the statistically significant difference between the 0.35 mg/kg dose group and the placebo at 12 hours was discounted even though the point estimate of reduction from baseline plasma ChE was > 10%.
- ***Male RBC ChE:*** there was no evidence of acephate effect on the blood RBC ChE, even at the highest dose level 1.25 mg/kg (note that the percent change from baseline RBC ChE of the 1.25 mg/kg at 12 hours was significantly different from that of the placebo group; however, the point estimate of RBC ChE reduction was <10%).
- ***Female Plasma ChE:*** there was evidence of acephate effect on the blood plasma ChE in the dose group 1.0 mg/kg (the percent change from baseline was significantly different

from the placebo at 8, 12, and 24 hours; point estimates of reduction from baseline plasma ChE were > 10% at 8, 10, and 12 hours). ***Female RBC ChE:*** there was no evidence of acephate effect on the blood RBC ChE in the dose group 1.0 mg/kg.

Conclusion

In conclusion, CEB identified issues with the registrant's approach and performed a more robust statistical analysis using mixed models. In terms of statistical significance, the results of both the registrant's analysis and CEB's analysis were similar. OPP will not use the ChE data from this study to establish a NOAEL or LOAEL. Rather, the results of CEB's analysis effectively reproduce the results of the registrant's statistical analyses and thus serve as positive confirmation of the quality of the data for other regulatory purposes. If AChE data would be considered in the future, CEB recommends that only the initial ChE measurement for each subject be used.

Appendix II

Calculations for the recovery of acephate and methamidophos in urine (%AD calculations) from Table 8.

Male Subject	dose (mg/kg)	wt (kg)	mg dosed	ng dosed	acephate (mg)	acephate (ng)	methamidophos (mg-eq)	meth (ng)	metabolite (% AD)	metabolite (% recovered)	% acephate	% meth eq	Total
2	0.35	82.8	28.98	28980000	16.844	16844000	0.19339	193390	0.667322291	1.135091701	58.1228433	0.6673223	58.79017
3	0.35	67.4	23.59	23590000	8.369	8369000	0.08077	80770	0.342390844	0.955884006	35.476897	0.3423908	35.81929
5	0.35	70.5	24.675	24675000	9.717	9717000	0.12917	129170	0.523485309	1.31188066	39.3799392	0.5234853	39.90342
6	0.35	93.4	32.69	32690000	14.129	14129000	0.1955	195500	0.598042215	1.364794583	43.2211686	0.5980422	43.81921
7	0.35	94.8	33.18	33180000	20.28	20280000	0.23144	231440	0.697528632	1.128345938	61.1211573	0.6975286	61.81869
9	0.35	62.7	21.945	21945000	5.494	5494000	0.07085	70850	0.322852586	1.273169987	25.0353156	0.3228526	25.35817
11	0.35	72	25.2	25200000	9.354	9354000	0.12469	124690	0.494801587	1.315477139	37.1190476	0.4948016	37.61385
min									0.322852586	0.955884006	25.0	0.32	25.4
max									0.697528632	1.364794583	61.1	0.70	61.8
4	0.7	63.9	44.73	44730000	22.896	22896000	0.35037	350370	0.783299799	1.50720306	51.1871227	0.7832998	51.97042
12	0.7	81.8	57.26	57260000	30.164	30164000	0.47491	474910	0.829392246	1.550022504	52.679008	0.8293922	53.5084
15	0.7	74.2	51.94	51940000	24.662	24662000	0.34653	346530	0.667173662	1.385647217	47.4817097	0.6671737	48.14888
16	0.7	60.5	42.35	42350000	22.615	22615000	0.24892	248920	0.587768595	1.0887022	53.4002361	0.5877686	53.988
18	0.7	60.7	42.49	42490000	23.772	23772000	0.2878	287800	0.677335844	1.196186169	55.9472817	0.6773358	56.62462
20	0.7	79.2	55.44	55440000	32.686	32686000	0.45193	451930	0.815169553	1.363784642	58.9574315	0.8151696	59.7726
21	0.7	60.9	42.63	42630000	11.778	11778000	0.13566	135660	0.318226601	1.138692895	27.6284307	0.3182266	27.94666
min									0.318226601	1.0887022	27.6	0.32	27.9
max									0.829392246	1.550022504	59.0	0.83	59.8
17	1	76.2	76.2	76200000	44.458	44458000	0.47158	471580	0.618871391	1.04959806	58.343832	0.6188714	58.9627
23	1	77.8	77.8	77800000	35.024	35024000	0.387	387000	0.497429306	1.092880743	45.0179949	0.4974293	45.51542
25	1	79.9	79.9	79900000	45.651	45651000	0.62439	624390	0.78146433	1.349291708	57.135169	0.7814643	57.91663
26	1	78.4	78.4	78400000	45.419	45419000	0.71535	715350	0.912436224	1.550579991	57.932398	0.9124362	58.84483
27	1	70.8	70.8	70800000	25.151	25151000	0.36749	367490	0.519053672	1.440093046	35.5240113	0.5190537	36.04306
29	1	83.7	83.7	83700000	50.745	50745000	0.6022	602200	0.719474313	1.172800075	60.6272401	0.7194743	61.34671
31	1	73.8	73.8	73800000	34.69	34690000	0.54028	540280	0.732086721	1.533567147	47.0054201	0.7320867	47.73751
min									0.497429306	1.04959806	35.5	0.50	36.0
max									0.912436224	1.550579991	60.6	0.91	61.3
28	1.25	67.6	84.5	84500000	44.336	44336000	0.72361	723610	0.856343195	1.605894947	52.4686391	0.8563432	53.32498
32	1.25	78.9	98.625	98625000	34.801	34801000	0.60806	608060	0.616537389	1.717244118	35.286185	0.6165374	35.90272
33	1.25	78.3	97.875	97875000	44.777	44777000	0.80512	805120	0.822600255	1.766306613	45.7491699	0.8226003	46.57177
34	1.25	74	92.5	92500000	39.709	39709000	0.82918	829180	0.896410811	2.045429765	42.9286486	0.8964108	43.82506
35	1.25	54.7	68.375	68375000	29.252	29252000	0.56063	560630	0.819934186	1.880511716	42.7817185	0.8199342	43.60165
37	1.25	71	88.75	88750000	39.605	39605000	0.54146	541460	0.610095775	1.348711692	44.6253521	0.6100958	45.23545
40	1.25	62.2	77.75	77750000	30.994	30994000	0.51163	511630	0.658045016	1.623931977	39.8636656	0.658045	40.52171
min									0.610095775	1.348711692	35.3	0.61	35.9
max									0.896410811	2.045429765	52.5	0.90	53.3

Female Subject	dose (mg/	wt (kg)	mg dosed	ng dosed	acephate (mg)	acephate (ng)	methamidophos (mg-eq)	meth (ng)	metabolite (% AD)	metabolite (% recovered)	% acephate	% meth eq	Total
41	1	66.9	66.9	66900000	15.872	15872000	0.23792	237920	0.355635277	1.476854013	23.7249626	0.35563528	24.0806
42	1	51.3	51.3	51300000	18.352	18352000	0.22514	225140	0.438869396	1.211919596	35.7738791	0.4388694	36.21275
43	1	81.6	81.6	81600000	9.835	9835000	0.14857	148570	0.182071078	1.488145022	12.0526961	0.18207108	12.23477
45	1	53.6	53.6	53600000	15.259	15259000	0.20997	209970	0.391735075	1.357362513	28.4682836	0.39173507	28.86002
47	1	52.6	52.6	52600000	7.011	7011000	0.0897	89700	0.170532319	1.263255735	13.3288973	0.17053232	13.49943
49	1	79.5	79.5	79500000	12.451	12451000	0.12511	125110	0.157371069	0.994822723	15.6616352	0.15737107	15.81901
50	1	78.6	78.6	78600000	39.798	39798000	0.54332	543320	0.691246819	1.34680769	50.6335878	0.69124682	51.32483
min									0.157371069	0.994822723	12.1	0.16	12.2
max									0.691246819	1.488145022	50.6	0.69	51.3

Appendix III. Study Selection Criteria.

Inclusion/exclusion criteria, pre-study screening parameters, restrictions, and actions resulting in removal from the study were detailed in the study report (MRID 45388301, starting on page 30 of the PDF) and are provided below.

Inclusion criteria:

To be entered into the study, subjects had to fulfill the following criteria:

(a) Males and females 18-50 years of age.
(b) No clinically important abnormal physical findings at screening examination.
(c) No clinically relevant abnormalities in the results of laboratory screening evaluation including plasma and RBC cholinesterase (Appendix C of the protocol).
(d) Normal ECG.
(e) Normal arterial pressure (BP) and heart rate (HR). Normal BP was taken to be 100 to 150 mm Hg systolic and 50 to 90 mm Hg diastolic. Normal HR was taken to be 50 to 90 beats per minute (b.p.m.). Normal erect HR (after standing for 1 min) was taken to be 50 to 100 b.p.m.
(f) Body weight between 50 and 100 kg and within +/- 15% ideal body weight as shown in Appendix D of the protocol (pg 154-155 of study report PDF).
(g) Able to communicate well with the study investigator and to comply with the requirements of the entire study.
(h) Provision of written informed consent to participate as shown by a signature on the volunteer consent form.

Exclusion criteria:

Any of the following would exclude a subject from the study:

(a) Administration of any investigational drug (or other test compound) in the period 0-3 months before entry to the study (0-4 months if previous investigational drug or other test compound was a new chemical entity).
(b) A need for any medication during the period 0-5 days before entry to the study.
(c) Existence of any surgical or medical condition which, in the judgement of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the test compound.
(d) Presence or history of allergy requiring treatment.
(e) Donation or loss of greater than 400 mL of blood in the period 0-12 weeks before the study.
(f) Serious adverse reaction or hypersensitivity to any drug.
(g) Inability to communicate or co-operate with the investigator because of a language problem, poor mental development, or impaired cerebral function.
(h) Objection by the subject's general practitioner to his/her patient's participation in the study.
(i) Females of childbearing potential who were not taking adequate contraceptive precautions.
(j) Females with a positive urine pregnancy test.

(k) Smokers who could not abstain from smoking from 2 h pre-dose to 8 h post dose.
(l) Any subject with a resting pulse of <45 b.p.m., a systolic BP of <100 mm Hg or a PR interval on ECG of >210 ms.
(m) Any subject who had exposure to anti-cholinesterases (including home pest control products) within one month of dosing.
(n) All agricultural workers or pest control applicators.

Pre-study screen:

The screening examination consisted of:
1. Medical history.
2. Complete physical examination and vital signs (pulse rate, respiratory rate and blood pressure).
3. 12-lead ECG recording.
4. Haematology, clinical chemistry, plasma and RBC cholinesterase and urinalysis.
5. Hepatitis B: Hbs-Ag
   Hepatitis C: Hep Cab.
   HIV infection: HIV antibody
6. Urine screening for drugs, including drugs of abuse (including cannabis).

Restrictions:

Volunteers were not permitted alcohol or other drugs whilst resident in the clinic and were advised to limit their alcohol intake to no more than 2 units daily until after the last blood sample had been withdrawn on Day 14. Caffeine was not permitted whilst the volunteers were resident within the clinic. No concomitant medications (apart from paracetamol or other medications used to treat adverse events) were allowed from 5 days before the study start or during the study unless approved by the Study Director.

Actions resulting in Removal:

A subject could be withdrawn from the study in any of the following circumstances:
1. Serious adverse events.
2. Major violation of the protocol.
3. Withdrawal of consent.
4. Termination of the study by the Sponsor.

No subject was withdrawn or withdrew from the study.