

REPORT TITLE

**A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY WITH
MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND RBC
CHOLINESTERASE ACTIVITY**

DATA REQUIREMENTS

Not applicable

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REPORT COMPLETION DATE

20 March 2000

SPONSOR

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LABORATORY PROJECT ID

ICR 013177

Volume 1 of 3

2. STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

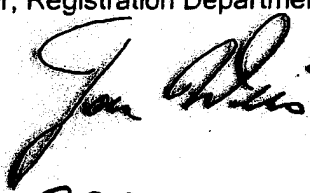
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B) or (C).

Company: Cheminova Agro A/S

Company Agent: Jon Weis

Title: Manager, Registration Department

Signature:



Date: 30 March 2000

These data are the property of Cheminova Agro A/S and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any statute or in any other country.

3. GOOD CLINICAL RESEARCH/LABORATORY PRACTICE STATEMENT

The study reported herein, was performed in compliance with the Principles of Good Clinical Practice (CPMP/ICH/135/95), OECD Principles of Good Laboratory Practice, and US EPA 40CFR Part 160, Good Laboratory Practice Standards and is consistent with the US EPA's 40CFR Part 26 (the Common Rule).

Deviations from the protocol are listed in Section 10.1.3. In the opinion of the Study Director, none of these deviations compromised the validity of this study.

This report is an accurate and authentic representation of the conditions and results of this study.

S. Freestone

Study Director

Dr. S. Freestone MD, FRCPedin

Inveresk Research

Date 24 March 2000

C.F. Wilkinson

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Authorised Representative of Cheminova Agro A/S

Date April 12, 2000

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4. STUDY SYNOPSIS

Study title	A Randomised Double-Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and Red Blood Cell Cholinesterase Activity
Name of sponsor	Cheminova Agro A/S, Denmark
Name of test compound	Malathion
Study centre	Inveresk Clinical Research
Study director	Stephen Freestone, MD
Duration of study period	October 15, 1998, first subject enrolled. March 22, 1999, dosing of last subject completed.
Objectives	To determine a No Observed Effect Level (NOEL) for plasma and red blood cell cholinesterase inhibition in healthy adult male and female volunteers.
Methodology	A randomised double-blind ascending single oral dose study.
Number of subjects	Forty eight subjects completed the study. Fifty one subjects initially entered into the study and three were replaced before the start of the study.
Main criteria for inclusion	Healthy males and females aged 18-50 years, weighing 50-100 kg and able to give written informed consent.
Main criteria for exclusion	Administration of any test compound 0-3 months, before entry into the study; Continuous need for medication; History of disease that might interfere with the results of the study or endanger the subject; Objection by the subject's General Practitioner; Females who were pregnant or were of childbearing potential but were not taking contraceptive precautions Smokers who could not abstain from smoking from 2 hours pre-dose to 8 hours post-dose.
Test compound batch	Malathion, Batch No. 50913-01 Placebo capsules Batch No. 56J2 and Batch No. 2881.
Dose and mode of administration	Administered as single oral doses of 0.5, 1.5, 5, 10, or 15 mg.kg ⁻¹ bodyweight in gelatin capsules.

Duration of observation	Four pre-dose visits, 3 nights residence in the clinic and 3 post-dose outpatient visits.
Evaluation criteria	Inhibition of plasma and red blood cell (RBC) cholinesterase (ChE) and occurrence (incidence and severity) of test material-related adverse events.
Parameters evaluated	Vital signs, continuous single channel ECG monitoring, haematology, clinical chemistry, plasma and RBC ChE, urinalysis, physical examination, adverse events, and concentration of malathion and/or its metabolites in blood plasma.
Statistical methods	<p>Percentage change from baseline values for plasma and RBC ChE activities were analysed separately for males and females using a repeated measures analysis of variance in terms of dose level, timepoint and dose level by timepoint interaction.</p> <p>For the male subjects, a test for linear trend using a linear contrast was carried out, and pairwise comparisons of plasma and RBC ChE were made between placebo and each dose level. If the test for linear trend was not significant at the 5% level, a Bonferroni adjustment was applied to the pairwise comparisons at each timepoint; otherwise no adjustment was made for multiple comparisons. For the females, comparisons of plasma and RBC ChE were made between placebo and treated subjects.</p> <p>Adverse events, vital signs, and other laboratory parameters were summarised descriptively by gender, dose level, and timepoint (where appropriate).</p>
Conclusion	<p>Results:</p> <p>Malathion was tolerated well from 0.5 mg.kg⁻¹ bodyweight to 15 mg.kg⁻¹ bodyweight in male subjects and at a dose of 15 mg.kg⁻¹ bodyweight in female subjects.</p> <p>No clinically significant changes in vital signs, ECGs haematology, clinical chemistry, urinalysis and physical parameters were observed in any subjects during the study. Furthermore, there were no adverse clinical or other effects of malathion in any subject and no statistically significant reductions in plasma or RBC ChE activity in male or female subjects. Consequently, a minimum NOEL for malathion given as a single oral dose to healthy</p>

male and female human subjects is 15 mg.kg⁻¹ bodyweight.

Pharmacokinetics:

Plasma samples from all male and female subjects dosed with malathion at 15 mg.kg⁻¹ bodyweight or placebo were analysed for malathion and malaoxon. Levels of malathion and malaoxon in these plasma samples were below the limits of quantification (<102 ng.ml⁻¹ and <99.8 ng.ml⁻¹ for malathion and malaoxon, respectively). Because 15 mg.kg⁻¹ bodyweight was the highest dose level of malathion administered, plasma samples from lower dose groups were not analysed.

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6. LIST OF ABBREVIATIONS

The following are taken from 'Units, Symbols and Abbreviations': A Guide for Biological and Medical Editors and Authors, printed by Royal Society of Medicine Services Limited, London, England.

b.p.m.	=	beats per minute
ECG	=	electrocardiogram
mmHg	=	millimetres of mercury
°C	=	degrees celsius
h	=	hours
min	=	minutes
s	=	seconds
kg	=	kilogrammes
g	=	grammes
mg	=	milligrammes
µg	=	microgrammes
ng	=	nanogrammes
mol	=	moles
µmol	=	micro moles
m	=	metres
cm	=	centimetres
mm	=	millimetres
l	=	litres
ml	=	millilitres
mg.l ⁻¹	=	milligrammes per litre
mg.kg ⁻¹	=	milligrammes per kilogramme (bodyweight)
µg.ml ⁻¹	=	microgrammes per millilitre
QAU	=	Quality Assurance Unit
ICR	=	Inveresk Clinical Research
IR	=	Inveresk Research
RBC	=	Red Blood Cell
ChE	=	Cholinesterase
ng.ml ⁻¹	=	nanogrammes per millilitre
Iu/l	=	international units/litre

7. INTRODUCTION

At the request of Cheminova Agro A/S, Inveresk Clinical Research (ICR) and Inveresk Research (IR) has carried out this oral dosing study on malathion, an organophosphorus insecticide that has been used for many years for agricultural pest control on a wide range of plants and crops in many parts of the world. It is also widely used in public health for control of insect vectors of disease and is a constituent of lotions and shampoos used in the treatment of scabies and head lice. As a result of these uses, accidental and incidental exposure of workers occurs and the public may be exposed to residues of malathion in food and water as well as from medicinal, residential and/or garden uses. The primary objective of this study was to determine the highest dose of malathion causing no effect or the lowest dose causing a slight inhibitory effect on blood cholinesterase activity in humans. This information will be used to provide a more accurate assessment of the margin of safety associated with anticipated human exposures. The study was conducted under carefully controlled clinical conditions with fully informed volunteers. The aim was to detect the effects of single oral doses of malathion on humans and identify a dose level with no effect on plasma and red blood cell (RBC) cholinesterase (ChE) activity.

Previous studies have established that malathion is rapidly absorbed after oral administration and almost entirely excreted within 24 h. In laboratory animals, malathion is of moderate to low acute toxicity with oral LD₅₀ values of 1000 mg.kg⁻¹ and greater. The main effect is inhibition of ChE activity.

The proposed doses of 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹ were considered unlikely to have measurable effects on blood ChE activity, and are very much lower than doses expected to cause any clinical signs.

The study protocol (Appendix A) was reviewed and approved by an Independent Research Ethics Committee (Appendix B) of IR. The constitution of the Independent Research Ethics Committee is attached in Appendix B. Following protocol approval by the Independent Research Ethics Committee, healthy volunteers were recruited from the surrounding area through a generic advertisement for volunteer participation.

The eligibility screening of the volunteers was conducted at ICR within 3 weeks of study initiation. Urine and blood samples were taken for urinalysis and routine haematology and clinical chemistry testing. During the screening visit, the scope and intent of the study were clearly explained to the volunteers by the screening physician. All participants were informed verbally and in writing about the objectives, procedures and risks involved in participating in the study and were clearly informed that the test compound was a pesticide. Following the screening process, and prior to being enrolled in the study, all participating volunteers read and signed informed consent forms written in English (Appendix B).

The study was conducted by physicians, a supportive staff of registered nurses, and a pharmacist. The study was conducted according to ICR's study plan for evaluating compound safety and appropriate GCP and GLP standards. After dosing, the volunteers stayed in the clinic for 3 days. During the conduct of the study, the attending physician/nurses explained the procedures for each phase of the study, and asked each volunteer if he/she had any questions on any procedures to be used, or any complaints associated with the administration of the chemical. A physical examination was performed prior to discharge from the clinical unit and all volunteers returned to the clinical unit 4, 7 and 14 days post-dose for blood sampling and adverse event reporting.

A physician was responsible for the care of the participants throughout the study. Blood samples were collected by registered nurses. During the course of the study, a study investigator kept the Independent Research Ethics Committee informed about any changes in the study activities. The study protocol required any unanticipated problems involving risks to the subjects to be immediately reported to the Chairman of the Independent Research Ethics Committee. With the exception of administrative Amendments 3 and 4, no changes were made in the study design without the Independent Research Ethics Committee's approval.

The clinical phase of the study was audited by the Quality Assurance Unit (QAU) of IR to assess adherence to the study protocol and appropriate GCP Standards. At the end of the study, the clinical report was audited by IR's QAU. A certifying statement, stating that the clinical report has been audited, is provided in the ICR clinical report (Appendix D).

8. STUDY OBJECTIVES

The study was undertaken in male human volunteers to establish an acute, single dose, oral No Observed Effect Level (NOEL). This was defined as the highest dose tested at which no inhibition of plasma or RBC ChE activity occurred and no clinical adverse effects were observed. A group of female volunteers was also to be studied at the NOEL identified in males.

9. METHODS AND INVESTIGATIONAL PLAN

9.1 INVESTIGATORS AND ADMINISTRATIVE STRUCTURE

The clinical phase of the study was carried out at ICR, Edinburgh and the study director was Dr. S. Freestone.

A list of other personnel involved in the study, including their qualifications and curricula vitae are presented in Appendix C.

The management and reporting of the study were the responsibility of ICR. Clinical pathology, analysis of the plasma samples for malathion and malaoxon and associated data management and quality assurance were the responsibility of IR.

Pacific Toxicology Laboratories (PTL), USA, conducted the analyses of urine samples for malathion metabolites. These data will be submitted in a separate report prepared by PTL.

The Sponsor, Cheminova Agro A/S provided the test compound, malathion. The matching placebo for the study, lactose, was provided by ICR.

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of IR for 15 years after issue of the final report, but after 5 years the Sponsor will be consulted regarding the continued storage of raw data.

9.2 STUDY DESIGN AND PLAN

The study was conducted in accordance with the final protocol and Amendments 1, 2, 3 and 4 (Appendix A).

The study was a randomised, double blind, ascending single oral dose study with malathion in healthy male and female volunteers. The study design was similar to that used routinely by ICR for evaluating drug safety.

The study involved 5 doses (0.5, 1.5, 5.0, 10.0 and 15 mg.kg⁻¹) and 48 subjects (38 males and 10 females) (Table 1).

TABLE 1

Dose Levels of Malathion Administered

	Placebo	0.5 mg.kg ⁻¹	1.5 mg.kg ⁻¹	5.0 mg.kg ⁻¹	10.0 mg.kg ⁻¹	15.0 mg.kg ⁻¹
Session 1 Subject 001-004 ^a	1	3				
Session 2 Subject 005-008 ^a	1		3			
Session 3 Subject 009-018 ^a	3			7		
Session 4 Subject 019-022 ^a	1				3	
Session 5 Subject 023-031 ^a	2				4	3
Session 6 Subject 032-038 ^a	3					4
Session 7 Subject 039-048 ^b	3					7

a = Sessions 1-6, males

b = Session 7, females

The study was conducted in 7 treatment blocks. In the first block one subject received placebo and 3 subjects received the lowest dose of malathion (0.5 mg.kg⁻¹).

The second treatment block also consisted of 4 subjects. One subject received placebo and 3 subjects received 1.5 mg.kg⁻¹ of malathion.

Session 3 consisted of 10 subjects. Seven subjects received 5.0 mg.kg⁻¹ of malathion and 3 subjects received placebo.

Session 4 consisted of 4 subjects. One subject received placebo and 3 subjects received 10.0 mg.kg⁻¹ malathion

Session 5 consisted of 9 subjects. Since no effect was seen at 10.0 mg.kg⁻¹ of malathion in Session 4, a further 4 subjects received 10.0 mg.kg⁻¹, 3 subjects received 15.0 mg.kg⁻¹ and two received placebo.

Session 6 consisted of 7 subjects. Since no effect was seen at 15.0 mg.kg⁻¹ in Session 5, a further 4 subjects received 15.0 mg.kg⁻¹ of malathion and 3 subjects received placebo.

Session 7 consisted of 10 female subjects, 7 of whom received 15.0 mg.kg⁻¹ of malathion and 3 placebo. Since the NOEL in males was greater than 15.0 mg.kg⁻¹, the highest level tested, the female group was dosed at 15.0 mg.kg⁻¹.

9.3 RATIONALE OF THE STUDY DESIGN

The oral route was chosen as the route of administration as it is a major potential route of human exposure to malathion. The doses were chosen after consideration of available data from the earlier animal and human studies.

9.3.1 Criteria for Stopping Study

The study would have been discontinued if any subject exhibited greater than 25% inhibition from baseline of RBC ChE or plasma ChE at two consecutive timepoints, or if, in any cohort, a mean inhibition greater than 15% was noted at two consecutive timepoints.

9.3.2 Criteria for Dose Escalation

Progression to the next higher dose level was permitted only after full review of all safety data indicated that it was safe to do so.

Dose escalation would not have occurred if 1) any subject had shown any signs or symptoms of organophosphorus toxicity, 2) if any subject had ≥25% inhibition

from baseline of RBC ChE or plasma ChE at two consecutive timepoints without associated symptoms or signs or 3) if in any cohort a mean inhibition of $\geq 15\%$ at two consecutive timepoints was noted.

9.4 ETHICS

9.4.1 Ethics Review Process

The protocol and volunteer information were submitted for the consideration of the IR Independent Ethics Review Committee.

The committee met on 11 August, 1998, reviewed draft 1 of the protocol, and agreed that it was ethically acceptable for the study to proceed provided several points raised were addressed. This included the revision of the study design, which was required to incorporate a single cohort protocol style.

Draft 2 was reviewed by the chairman of the committee, Dr. T. M. Chalmers, on 13 October, 1998, and again approval was granted provided that the points raised were addressed. This included the incorporation of a criteria for dose escalation be set so that dose escalation would not occur if there were any significant inhibition of ChE activity.

The final protocol was reviewed and approved by the chairman of the committee on the 28 October, 1998.

Protocol amendments 1 and 2 were submitted to the chairman of the Ethics Committee and were approved on the 4 November, 1998, and 29 January, 1999, respectively. Amendment 1 was issued to incorporate changes required by the Sponsor, this included a new section on Analysis of Plasma Samples. Amendment 2 was issued to clarify the dose level, that the female subjects would receive and also to document that the urine analysis would now be done by Pacific Toxicology Laboratory.

Protocol amendments 3 and 4 did not require approval by the Ethics Committee as they involved only administrative changes. Amendment 5 was an

administrative amendment documenting that the urine analysis would be reported separately from the IR report. As this was an administrative amendment, it did not require approval by the Ethics Committee. The final protocol and its amendments are presented in Appendix A. Details of Ethics Committee approvals including amendments are presented in Appendix B.

The study was conducted in accordance with the guidelines set out in the Declaration of Helsinki, 1964, as amended by the 29th World Medical Assembly in Tokyo, 1975, the 35th World Medical Assembly in Venice, 1983, the 41st World Medical Assembly in Hong Kong, 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996.

9.4.2 Subject Information and Consent

At screening, each subject was informed of the nature and risks of the study and given a copy of the volunteer consent form and information to review. On admission to the clinic on the evening prior to dosing, written informed consent was obtained from each volunteer. Each subject's general practitioner was asked if they had any objections to their patient's participation before the start of the study. This form is shown in Appendix B of the protocol.

9.5 SELECTION OF STUDY POPULATION

Subjects were healthy male and female volunteers selected from the panel of volunteers recruited by ICR. A total of 48 subjects completed the study.

Up to 21 days before dosing, subjects were screened for inclusion in the study according to the criteria for inclusion.

9.5.1 Inclusion Criteria

- (a) Males and females 18-50 years of age.
- (b) No clinically important abnormal physical findings at the screening examination.

- (c) No clinically relevant abnormalities in the results of laboratory screening evaluation including plasma and RBC ChE (Appendix C of the protocol).
- (d) Normal ECG.
- (e) Normal arterial pressure (BP) and heart rate (HR). These were measured after resting supine for 3 min. Normal BP was taken to be 100-150 mmHg systolic and 50-90 mmHg diastolic. Normal HR was taken to be 50-90 b.p.m. Erect heart rate was measured after standing for 1 min. Normal erect HR was taken to be 50-100 b.p.m.
- (f) Body weight between 50 and 100 kg and within $\pm 15\%$ of ideal body weight as shown in Appendix D of the protocol.
- (g) Able to communicate well with the 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.

9.5.2 Exclusion Criteria

- (a) Administration of any investigational test compound in the period 0-3 months before entry to the study (0-4 months if the previous investigational 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, may have interfered 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 entry to 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 pregnancy test.
- (k) Smokers who could not abstain from smoking from 2 h pre-dose to 8 h post-dose.
- (l) Any subject who had a resting pulse of <45 b.p.m., a systolic BP of <100 mmHg or a PR interval on ECG of >210 ms.
- (m) Any subject who had been exposed to an anti-ChE (including home pest control products) within one month of dosing.
- (n) All agricultural workers or pest control applicators.

9.5.2.1 Pre-study screen

The screening examination consisted of the following:

1. Medical history.
2. Complete physical examination and measurement of vital signs (pulse rate [supine and erect], respiratory rate, blood pressure and oral temperature).
3. 12-lead ECG recording.
4. Haematology, clinical chemistry, plasma and RBC cholinesterase and urinalysis.
5. Hepatitis B: Hbs-Ag.
HIV infection: HIV antibody.
Hepatitis C: Hep C Ab.
6. Urine screening for drugs, including drugs of abuse (including cannabis).
7. Pregnancy test (the cohort of females).

Restrictions: No alcohol, caffeine or concomitant medications (except those prescribed to treat adverse events) were allowed during the study.

9.5.3 Removal of Subjects from Study

Subjects wishing to leave the study at any time were permitted to do so.

A subject could withdraw or have been withdrawn from the study if any of the following circumstances had occurred:

1. Serious adverse event.
2. Major violation of the protocol.
3. Withdrawal of consent.
4. Termination of the study by the Sponsor.

Any subject discontinuing the study prematurely because of reasons 1 or 4 would be considered to have completed the study. If subjects had withdrawn because of reasons 2 or 3 they would have been replaced.

If a subject had withdrawn for non-medical reasons at short notice, the following procedure would have been adopted:

- The medical risks of withdrawing from the study within 12 h of dosing would have been explained to the subject.
- A physical examination would have been performed.
- The 24 h post-dose safety assessments (vital signs, ECG, haematology, clinical chemistry and urinalysis) would have been performed.
- The subject would have signed a 'Discharge Against Medical Advice' form if necessary.

9.6 TREATMENTS

9.6.1 Identity of the Test Compound

The test compound was provided by the sponsor as a clear liquid, in a glass bottle to be stored in the dark at ambient temperature (15-25°C). The matching placebo, lactose, was provided by ICR.

The clinical trial supplies (test compound and placebo) were received at IR on 31 July, 1998 and stored in dark ambient conditions before transfer to ICR pharmacy on 13 August, 1998. They were then stored under the control of the

ICR pharmacist in a locked, dark cupboard in the pharmacy at ambient temperature (15-25°C).

The bottles containing capsules of test compound or placebo were labelled with the Sponsor's name, study number, subject number and dosing details.

An accountability record of utilisation of the test compound and placebo was maintained throughout the study.

The test compound was malathion, Batch Number: 50913-01, CAS Number: 121-75-5, Purity: 95.8% w/w, Expiration Date: 9 March, 1999. The Certificates of Analysis are given in Appendix F. The malathion remaining at the end of the study was returned to Cheminova Agro A/S for reanalysis and was received on the 25 June 1999. Compared with the results of an analysis conducted in September 1998, the June 1999 analysis showed that the content of malathion had decreased from 95.8% w/w to 95.4% w/w.

9.6.2 Assignment of Subjects to Treatment Groups

A number was assigned to all subjects who qualified for the study in accordance with exclusion and inclusion criteria. The number assigned was the lowest number available. Subject numbers were allocated according to the code 001-099, replacement subjects were identified by 901-999. Based upon a computer generated randomisation, subjects were assigned to one of the dose levels, or placebo.

The randomisation code was kept confidential and was held by the Regulatory Affairs Department at IR and the Pharmacy Department at ICR where it was required for dispensing purposes.

9.6.3 Blinding

This was a double blind, placebo controlled study. The allocation of subjects to a treatment group or to placebo was random and based on the randomisation code generated by the statistics department of IR.

A copy of the randomisation code was held by the ICR pharmacist who required it for dispensing purposes. Separate copies were sent to the Regulatory Affairs Department of IR. It was the responsibility of IR to ensure that blinding was maintained during the study. The test compound and placebo were packaged in identical capsules to ensure that treatments were indistinguishable, thus retaining study blindness.

Sealed disclosure envelopes were also provided to ICR. In the event of an emergency requiring identification of the test compound administered to a subject, the study director could request that the envelope be opened. This did not occur.

9.6.4 Treatment Administered

The treatment, a single, oral dose of test compound or placebo in a gelatine capsule was administered to subjects in the sitting position with 150 ml of water approximately 5 min after completing a light breakfast. The test compound was malathion and the matching placebo was lactose. Doses were prepared by direct weighing of the malathion liquid or placebo into size zero hard gelatine capsules.

The target weight of malathion or placebo in each capsule was based on the dose level desired and the body weight of the individual volunteer at screening. Weighing of malathion or placebo into capsule shells was undertaken on a maintained and calibrated electronic balance with a readability capability of 0.01 mg. Capsules were filled to an accuracy of $\pm 5\%$ of target dose weight for each volunteer.

The weights of malathion or placebo transferred to each capsule shell for each volunteer were independently verified by the pharmacist who was responsible for the dose calculation.

9.6.5 Allowable Medications

No medications with the exception of paracetamol (acetaminophen) ions deemed necessary to treat adverse events were allowed during the study. Atropine sulphate and a ChE reactivator (pralidoxime; 2-PAM) were available for use in the highly unlikely event that cholinergic symptoms or signs were observed.

9.6.6 Treatment Compliance

Subjects were supervised by medical or nursing personnel whilst in the clinic. Dosing was performed by nursing staff who recorded in the Case Record Form (CRF) the date and time that each subject was dosed. Mouth checks were performed routinely for each subject immediately after dosing. A copy of the dispensing label was inserted into each subject's CRF on dosing day. Information on the dispensing label included the following: study number, subject number, weight of volunteer, dose level: mg.kg⁻¹, actual dose (of test compound or placebo), dosing instructions, expiration date and name of sponsor.

Any deviations from the protocol were recorded.

9.7 PARAMETERS EVALUATED

9.7.1 Vital Signs

Measurements for vital signs included supine systolic and diastolic arterial blood pressure (determined by sphygmomanometry), standing and supine pulse rates (determined by palpation), respiratory rate (determined manually), and oral temperature (determined using a mercury thermometer). These measurements were made for all subjects at screening, on day -1, pre-dose (0) and at 2, 4, 8, and 24 h after dosing.

9.7.2 Electrocardiography (ECG)

12-lead ECG tracings were obtained for all subjects at screening, at -30 minutes pre-dose, and 2, 4, 8, and 24 h after dosing. Any abnormality apparent at any

stage was confirmed by repeat tracings and abnormality followed to resolution. Additional lead recordings were taken as deemed necessary.

In addition, single channel continuous ECG monitoring was performed for all subjects using a bedside monitor from –30 minutes pre-dose to 4 h post-dose. Any subject demonstrating sustained bradycardia *i.e.* <50 b.p.m. or tachycardia *i.e.* >110 b.p.m. for more than 30 seconds would have been evaluated by the clinical investigator.

9.7.3 Laboratory Investigations

Blood samples were collected from all subjects in pre-heparised tubes (5.0 ml) for clinical chemistry tests and in EDTA-coated tubes (3.0 ml) for haematology tests. Laboratory tests were performed for all subjects at screening, pre-dose (0), and 24 h after dosing.

9.7.3.1 Haematology

All haematology measurements were performed using an automated analyser (Technicon H1 Analyser, Bayer UK Ltd). The following parameters were evaluated:

- Haemoglobin (Hb)
- Total red blood cell count (RBC)
- Haematocrit (Hct)
- Mean cell haemoglobin (MCH)
- Mean cell volume (MCV)
- Mean cell haemoglobin concentration (MCHC)
- White blood cell count (WBC)
- Differential white cell count
- Platelets

The normal ranges for males and females for all parameters with their appropriate units are provided in Appendix C of the Protocol. Abnormal results for any subject were confirmed by repeat analysis. Any abnormal

value fulfilling the common toxicity criteria listed in Appendix E of the protocol would have been treated as an Adverse Event.

9.7.3.2 Clinical Chemistry

All clinical chemistry measurements were performed using an automated chemistry analyser (ILAB900, IL UK Ltd).

The following parameters were evaluated:

- Urea
- Glucose (Glu)
- Aspartate amino-transferase (AST)
- Alanine amino-transferase (ALT)
- Lactate dehydrogenase (LDH)
- Sodium (Na)
- Potassium (K)
- Chloride (Cl)
- Total Protein (TP)
- Albumin (Alb)
- Creatinine (Crea)
- T. Bilirubin (T.Bil)
- Gamma glutamyl transpeptidase (GGT)

The normal ranges for males and females for all parameters with their appropriate units, and references for all methods are provided in Appendix A (Protocol-Appendix C). Abnormal results for any subject were confirmed by repeat analysis. Any abnormal value fulfilling the common toxicity criteria listed in Appendix E of the Protocol would have been treated as an Adverse Event.

9.7.3.3 Urinalysis

Urinalysis was performed on all subjects at screening and 24 h post-dosing. The following qualitative tests were performed using Mustistix[®] (Ames Division):

pH
Protein
Glucose
Ketones
Bilirubin
Blood
Urobilinogen
Specific Gravity

Any urine sample showing the presence of protein or blood was centrifuged and the sediments were examined microscopically.

Any abnormal value fulfilling the common toxicity criteria listed in Appendix E of the Protocol would have been treated as an Adverse Event.

9.7.4 Adverse Clinical Events

All observed or reported signs or symptoms were recorded and fully described with respect to their duration and severity (as defined by toxicity rating scales, Appendix E of the Protocol). An assessment was made with respect to possible treatment relatedness and details of any specific therapy required were recorded.

9.7.5 Blood Cholinesterase (ChE) Assay

In addition to the above-listed clinical chemistry parameters, blood samples were collected in EDTA-coated tubes from all subjects for evaluation of plasma and RBC ChE activity at the following intervals: screening, and on days -9, -7, -5, -2,

-1, and -30 minutes before dosing. When possible, all pre-dose samples were collected at about the same time in the morning.

The post-dosing samples were collected at 1, 2, 4, 8, 12, 24 and 48 h and on days 4, 7, and 14. ChE activity was analysed using the modified Ellman procedure included in Appendix P.

9.7.6 Pharmacokinetics/Drug Concentration Measurements

Plasma

Blood samples were collected for measurement of malathion and its metabolite malaoxon concentrations, in two 7 ml lithium heparin tubes either by cannula or by direct venepuncture at the following timepoints:

Pre-dose (0), 1, 2, 4, 8, 12, 24, 48 and 72 h post-dose

Plasma was obtained by centrifugation. Since a minimum of 5 ml of plasma was required for analysis, the plasma obtained from both blood tubes was pooled as one plasma sample and stored at -70°C until analysis. Samples were transferred for analysis in dry ice to:

D L Scott
Department of Bioanalytical Chemistry
Inveresk Research
Tranent, EH33 2NE
Scotland

The plasma samples were analysed for malathion and malaoxon concentrations using Analytical Method No. 6674. This method was validated, over the concentration range ca 100-1000 ng.ml⁻¹ plasma for each analyte, under Inveresk Project No. 366748 (Inveresk Report No. 17123) prior to the analysis of any samples.

In this method, aliquots of plasma (1.0 ml) were fortified with malathion and malaoxon in acetonitrile (50 µl) (for calibration standard and quality control samples). Internal standard (bromophos-ethyl) in acetonitrile (50 µl) was added and the samples were extracted by liquid/liquid extraction with toluene. The sample extracts were then evaporated to dryness and reconstituted in polyethylene glycol (average molecular weight 400) in acetone (0.02%). Aliquots (8 µl) of the final extracts are then analysed by gas chromatography with flame photometric detection (phosphorus mode).

The peak area ratios for malathion:internal standard and malaoxon:internal standard were calculated for each sample. A calibration curve was prepared for each compound by plotting peak area ratio of the standard samples vs the concentration of each compound in plasma and the weighted (1/concentration) least squares linear regression parameters of each standard curve calculated. The concentrations of malathion and malaoxon in the study samples and quality control samples were determined by interpolation from the appropriate calibration curve.

Urine

Urine was collected for measurement of malathion mono- and di-carboxylic acids and dimethyl phosphate, dimethyl thiophosphate and dimethyl dithiophosphate at the following times:

-12-0 h (pre-dose), 0-12, 12-24 and 24-48 h post-dose

Total urine volume was measured from each subject and sampling period and 2 x 20 ml aliquots from each sampling period were frozen at -70°C until analysis. Samples were transferred for analysis on dry ice to:

Pacific Toxicology Laboratories
 6160 Variel Avenue
 Woodland Hills
 CA 91367
 USA

TABLE 2

Schedule for Evaluation of Physiological/Clinical Parameters, Effects and Pharmacokinetic Sampling

	Pre-study	Pre-dose			Times After Dosing (h)								Post Study Days		
		Day -1	-30 min	0	1	2	4	8	12	24	48		4	7	14
Informed consent		x													
History	x														
Physical Examination	x										x				
Compound Admin				x											
Haematology	x			x						x					
Clinical Chemistry	x			x						x					
Urinalysis	x									x					
ECG (12 lead)	x		x			x	x	x		x					
Vital Signs	x	x		x		x	x	x		x					
Urine Drug Screen	x														
Virology	x														
Pregnancy Test (♀ Cohort)	x	x													
PK Blood Sample				x	x	x	x	x	x	x	x	x			
Cholinesterase Assay	x	x ^a	x		x	x	x	x	x	x	x	x	x	x	x
Adverse Events		←-----→													
Test Compound Accountability		←-----→													
Continuous Single Channel ECG			←-30 min to 4 h→												
Urine		←-12-0 h→		←0-12 h→				←12-24 h→		←24-48 h→					

a = Samples for plasma and RBC ChE assay were also collected on Days -9, -7, -5 and -2

9.8 QUALITY CONTROL/QUALITY ASSURANCE

Quality Assurance inspections were carried out during the clinical and reporting phases of the study. Phases selected for inspection included:

Dosing and protocol compliance
 CRF data review

Phases selected for audit included:

Data listings and tables

Draft report

Final report

Quality assurance inspections were carried out on critical phases in the execution of the study. Further inspections on routine, repetitive processes were also performed although not necessarily on elements from this study (Appendix D).

These inspections and audits were carried out according to the relevant Standard Operating Procedures by Quality Assurance personnel independent of the staff involved in the study. Records of these inspections and audits have been documented and distributed to management for review.

9.9 DATA MANAGEMENT AND STATISTICAL METHODS

9.9.1 Determination of Sample Size

The sample size of 48 subjects (4 to 10 subjects per dose level) is considered appropriate for a study of this type, following a power calculation, to minimise the exposure of the test compound to healthy volunteers.

9.9.2 Data Management Methods

Data management was performed by the Statistics and Data Management Department at IR.

Adverse events and medications were coded using the WHO Adverse Reaction Terminology (1997) and the WHO Drug Reference List (1997) respectively. Primary and secondary coding was performed and discrepancies adjudicated by an independent third party.

All study data recorded on the CRF, except clinical chemistry, haematology, RBC and plasma ChE were subjected to double data entry using a validated database programmed in Clintrial (v4.1), a clinical data management system. On completion of data entry, the data were exported to SAS for further consistency and validation checks. Following comparison of the data entries, the database

was closed and the audit trail switched on, *i.e.* a computerised log of all subsequent changes to the data was recorded. All data queries were raised using Data Resolution Forms (DRFs) and were resolved with the assistance of ICR medical staff.

On resolution of all data queries, the database was closed and all study data were exported to SAS (v6.09) for the production of data listings and summary tables.

Clinical chemistry, haematology, RBC and plasma ChE data were collected by the online clinical pathology system and electronically transferred to SAS for the production of summary tables and data listings.

Plasma concentrations of malathion and malaoxon were doubly entered into ASCII files. The resulting datasets were compared and the final dataset was reformatted in SAS and subjected to a 100% check against the source data. Furthermore plasma test compound and/or metabolite concentration data were reformatted via SAS to an ASCII file suitable for importing into WinNonlin (v1.1) (a pharmacokinetics modelling package). The reformatted data were entered in hours and all pharmacokinetic parameters were presented in terms of hours. Pharmacokinetic parameter values were received in the form of WinNonlin ASCII file output. This file was read into SAS.

All data listings for inclusion into the study report, except adverse events, were subjected to 10% quality control checks against the CRFs. All adverse events listings and summary tables were subjected to 100% quality control checks.

On issue of the final report, IR standard SAS datasets, used for the purposes of reporting and analysis, were made available to the Sponsor.

9.9.3 Statistical and Analytical Plans

The statistical package SAS (v6.09) was used to perform all statistical analyses and produce all summary tables and data listings. All statistical analyses were performed by the Statistics and Data Management Department at IR.

Throughout this section, data from the male subjects from the different dosing cohorts who received placebo were combined

In general terms, categorical data were presented using counts and percentages, whilst continuous variables were presented using the mean, standard deviation, minimum, maximum and number of subjects. In general, minima and maxima were quoted as whole numbers as recorded in the CRF; means and standard deviations were quoted to one further decimal place. Percentages were rounded to one decimal place.

9.9.3.1 Demographics and Other Baseline Characteristics

The following demographic variables were summarised by gender and dose level: race, age, height, weight and physical examination details (normal/abnormal by body system). No significance testing of demographic data was performed.

9.9.3.2 Clinical Parameters

The objective of the statistical analysis was to evaluate the data for any effects of test material on plasma and RBC ChE activities and clinical signs of toxicity. Those parameters were summarised with respect to gender, dose level and time after administration. With the exception of the RBC and plasma ChE, no formal statistical analyses were performed.

The results for RBC and plasma ChE activities were summarised (*i.e.* mean, standard deviation, minimum, maximum and *n*) at each time point, by gender and dose level. The percentage change of plasma and RBC ChE activity from baseline at each time point was calculated and tabulated by gender and dose level and illustrated graphically. Both individual and group values were evaluated. Baseline was defined as the mean of all available pre-dose values except screening (*i.e.* Days -9, -7, -5, -2, -1 and -30 min).

For the male data, percentage changes from baseline for RBC and plasma ChE were analysed using a repeated measures analysis of variance (ANOVA) including terms for dose level, time point (*i.e.* 1, 2, 4, 8, 12, 24, 48 h, and Day 4, Day 7 and Day 14 post-dose) and dose level by time point interaction. Subject was included as a random effect. At each time point separately, a test for linear trend with dose was performed using a linear contrast. In addition, using the error variance from the ANOVA pairwise comparisons between placebo and each dose level were carried out, at each time point, using Student's 't'-tests. At each timepoint, if the test for linear trend was significant at the 5% level then the pairwise comparisons at that time point were not adjusted for multiple comparisons. If the test for linear trend was not significant at the 5% level, a Bonferroni adjustment was applied to the pairwise comparisons at that time point (*i.e.*, each comparison was to be tested at the 1.7% significance level). Adjusted means (*i.e.*, means adjusted for any imbalance in the model) for each dose group were presented together with the significance level of each pairwise comparison and the test for linear trend. In addition, where a Bonferroni adjustment was applied, the significance level of the pairwise comparisons after adjustment was also presented.

For the female data, percentage changes from baseline for RBC and plasma ChE activities were analysed using a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (*ie*, 1, 2, 4, 8, 12, 24, 48 h, Day 4, Day 7 and Day 14 post-dose) and dose level by time point interaction. Subject was included as a random effect. Using the error variance from the ANOVA, a comparison between placebo and active group was carried out, at each time point, using a Student's 't'-test. Adjusted means (*ie*, means adjusted for any imbalance in the model) were presented for each dose group together with the significance level of the 't'-test.

Distributional assumptions underlying the statistical analyses were assessed as follows: Normality was examined using a Shapiro-Wilk test while homogeneity of variance was assessed by plotting the residuals against the predicted values for the model. If there was significant non-normality which could not be resolved by transforming the data, the data were analysed excluding outliers. However, if the omission of outliers had no effect on the conclusions, the results of the full dataset only were reported.

Vital signs were summarised (*i.e.*, mean, standard deviation, minimum, maximum and n) at each time point, including changes from baseline (*ie*, pre-dose), by gender and dose level. Additionally, the number of subjects with 'substantial' increases or decreases in blood pressure (> 20 mmHg) and heart rate (>15 b.p.m.) was tabulated.

Laboratory parameters were summarised at each time point including changes from baseline (*i.e.*, pre-dose), by gender and dose level. In addition, abnormal values outside normal ranges were flagged in the data listings.

The following data are presented by dose level and, where appropriate, timepoint:

Demographic details

ECG

Urinalysis

Adverse clinical events

Plasma concentrations of test compound and/or metabolite

Throughout the study, all adverse events (whether they were related to the test compound or not), were evaluated by the investigator and were noted in the adverse event section of the CRF.

Information relating to adverse events included all events (whether related to test compound or not) observed by the medical staff or reported by the subject. The adverse events were recorded in the CRF. The following information is provided in relation to both gender and dose level:

- the number and percentage of subjects with at least one adverse event;
- the number and percentage of subjects with serious adverse events;
- the number and percentage of subjects with possible test compound-related adverse events.

The following breakdown of adverse events was provided, by gender and dose level:

- by body system, preferred term and severity;
- by body system, preferred term and possible relationship to test compound.

Adverse events were coded using the WHO Adverse Reaction Terminology. Adverse events were reported by primary body system and preferred term. In the tabulations, counting was performed by subject and not event. That is a subject reporting the same event more than once had that event counted only once. All adverse events commencing prior to dosing with test compound were excluded from the summary tables but were fully listed.

9.9.3.3 Pharmacokinetic Methods

The protocol required that a measured test compound concentration vs time curve was to be produced, in graphic and tabular form, for each subject on both linear/linear and log/linear scales. Mean test compound concentration vs time curves were also to be presented for each dose

level separately. Summary statistics (*i.e.* mean, standard deviation, minimum, maximum, n and coefficient of variation) were to be calculated for plasma concentrations for each timepoint and each dose level.

Since no quantifiable levels of either malathion or malaoxon were measured in plasma of either male or female subjects at the highest dose employed, 15.0 mg.kg⁻¹ bodyweight and since plasma from subjects receiving lower doses were not analysed, the proposed pharmacokinetic evaluations were not conducted.

9.9.4 Changes in the Conduct of the Study or Planned Analyses

9.9.4.1 Changes in the Conduct of the Study.

The study was conducted according to the Protocol (Appendix A). None of the amendments to the protocol changed the conduct of the study.

9.9.4.2 Changes in the Planned Data Analyses

Clinical Analysis

Where the Bonferroni adjustment was applied to the pairwise comparisons at a particular timepoint, each comparison was tested at the 1% significance level. This change is due to the fact that 5 doses were investigated and not three as anticipated at the time of writing the original statistical methods.

Pharmacokinetic Analysis

Calculations for concentrations of malathion and malaoxon in human plasma were performed only on the male and female subjects, who received 15.0 mg.kg⁻¹ malathion or placebo. Since plasma concentrations of malathion and malaoxon were not quantifiable at this dose level (the highest dose tested), plasma samples from the remaining dose groups were not analysed.

10. **RESULTS**

10.1 **SUBJECT ACCEPTANCE AND TREATMENT**

A total of 51 subjects were recruited for the study and the planned total of 48 subjects, 38 males and 10 females, completed the study. The three extra subjects replaced Subjects 017, 027 and 048 who withdrew before dosing and were numbered 917, 927 and 948, respectively. Subjects 017 and 027 were unable to swallow the capsules containing the test compound and Subject 048 had screening and pre-dose plasma and RBC ChE levels below the normal ranges (Table S1).

10.1.1 **Demographic and Other Baseline Characteristics**

Demographic details are summarised by gender and dose level in Table H1. Individual values are listed in Tables H2.1.1 and H2.1.2. The mean (SD) age amongst male subjects was 33.4 (8.1) years. Generally, all dose groups were reasonably well balanced for age: the 15.0 mg.kg⁻¹ dose group had the lowest mean (SD) age of 30.1 (5.6) years and the 5.0 mg.kg⁻¹ dose group had the highest mean (SD) age of 35.5 (9.2) years.

The mean (SD) height amongst male subjects was 173.7 (5.8) cm; generally all dose groups were reasonably well balanced for height. The mean (SD) weight amongst male subjects was 72.22 (9.50) kg; generally mean weight varied over the dose groups. The 15.0 mg.kg⁻¹ dose group had the lowest mean (SD) weight of 66.16 (5.82) kg with a range of 60.8 to 73.0 kg and the 1.5 mg.kg⁻¹ dose group had the highest mean (SD) weight of 81.10 (3.84) kg with a range of 77.6 to 85.2 kg. The other 4 dose groups were reasonably well balanced for weight. The mean (SD) caliper size (elbow breadth) amongst male subjects was 7.29 (0.28) cm; generally all dose groups were reasonably well balanced for caliper size. All male subjects in the study were white.

The mean (SD) age amongst female subjects was 27.4 (6.3) years; the 15.0 mg.kg⁻¹ dose group had the higher mean (SD) of 28.0 (7.2) years and the placebo dose group had the lower mean (SD) age of 25.7 (3.2) years. The mean (SD) height and weight amongst female subjects was 167.4 (3.7) cm and 66.40

(8.77) kg; generally both dose groups were well balanced for height and weight. The mean (SD) caliper size (elbow breadth) amongst female subjects was 6.66 (0.30) cm; generally both dose groups were reasonably well balanced for caliper size. All female subjects were white.

10.1.2 Treatment with Test Compound

All subjects in the study received dose levels of malathion or placebo based on body weight at screening in accordance with the final protocol and its amendments. Dosing details, including the actual doses of malathion administered to individual subjects, are shown in Appendix G (Table G2) and Table 3. Among the males, 14 subjects received placebo, 3 subjects received 0.5 mg.kg⁻¹, 3 subjects received 1.5 mg.kg⁻¹, 7 subjects received 5 mg.kg⁻¹, 7 subjects received 10 mg.kg⁻¹ and 7 subjects received 15 mg.kg⁻¹. Among the 10 females, 3 subjects received placebo and 7 subjects received 15 mg.kg⁻¹.

10.1.3 Protocol Deviations

Two subjects (002 and 007) failed an inclusion criterion and one, Subject 047, failed an exclusion criterion (Table H2.5). Subjects 002 and 007 were underweight and Subject 047 took medication (contraceptive pill) during the 5 day period before entry into the study. The study director allowed these subjects to continue with the study since it was concluded that these failures would have no effect on the results observed. Other protocol deviations associated with acceptance of subjects into the study or treatment during the study are shown in Table 4.

TABLE 3**Actual Dose (mg) Received by Individual Subjects**

Subject No.	Dose Group	Actual Dose Received (mg)
1	0.5	37.59
2	0.5	26.51
3	Placebo	37.96
4	0.5	35.89
5	1.5	126.23
6	1.5	131.69
7	Placebo	120.79
8	1.5	112.07
9	5.0	329.08
10	5.0	447.85
11	5.0	339.92
12	5.0	358.60
13	Placebo	416.58
14	5.0	321.05
15	Placebo	325.37
16	Placebo	408.86
917	5.0	365.77
18	5.0	368.78
19	10.0	733.05
20	10.0	726.72
21	Placebo	574.63
22	10.0	654.04
23	10.0	821.20
24	Placebo	1026.96
25	Placebo	1064.35
26	15.0	1060.85
927	10.0	927.01
28	10.0	731.74
29	15.0	914.47
30	10.0	541.09
31	15.0	948.51
32	Placebo	886.76
33	15.0	925.48
34	15.0	1095.82
35	Placebo	962.34
36	Placebo	1114.89
37	15.0	1084.62
38	15.0	926.67
39	15.0	1148.93
40	15.0	829.76
41	Placebo	1169.96
42	15.0	1040.43
43	15.0	1082.56
44	15.0	953.29
45	Placebo	954.08
46	15.0	1024.61
47	Placebo	884.20
948	15.0	1064.30

TABLE 4**Protocol Deviations**

Subject Number	Protocol Deviation
For all subjects	The –30 min ChE blood sample was taken along with the pre-dose bloods with the approval of the Clinical Investigator.
For 9 subjects	The pre-dose vital signs were taken following the –30 min ECG. This was to allow the volunteers time to have their breakfast and allow 5 min prior to dosing for blood sampling.
Subject 013	This subject did not have an erect pulse done at screening in error.
Subject 917	This subject had finished his breakfast more than 5 min prior to dosing as per the protocol. This subject replaced subject 017 and the delay was due to organisation of the reserve subject. There was also a delay in commencing the 0-12 h urine collection.
Subject 014	This subject did not attend the clinic for his Day 4 outpatient visit due to a hospital admission, and his Day 4 ChE sample was not obtained. This subject's Day 4 PK blood sample was taken on Day 7 along with his Day 7 ChE sample. Approval for this was given by the Clinical Investigator.
Subject 031	Day 4 ChE and PK blood samples not taken as subject attended late and clinic was closed.
Subject 047	This subject screened 22 days prior to dosing and not 21 as specified in the protocol. This was because he initially screened to be used in a previous dose group but because he was unwell at that time he transferred to a later dose group.
For some subjects the pre-dose blood samples were not taken on the scheduled day.	

10.2 EFFECTS ON BLOOD CHOLINESTERASE (ChE)

The results of malathion treatment on plasma and RBC ChE levels in both male and female subjects are provided in Appendix M. In each case, the results are presented in relation to dose and time after dosing and both group mean (Tables M2.1-M2.2) and individual values (Table M3) are provided. Appropriate statistical analyses for dose related trends and pairwise comparisons with baseline levels were also conducted (Tables M1.1.1-M1.2.2). The results are summarised in Tables 5 and 6 (plasma ChE) and Tables 7 and 8 (RBC ChE). Baseline levels of ChE are defined as the means of all group or individual pre-dose values (from Day –9 to –30 min) except those obtained during initial screening. ChE values below and above baseline are expressed as a percentage of the baseline values in Tables 5-8. In these tables, positive values indicate ChE activity above the baseline while negative values indicate ChE activity below baseline.

TABLE 5

Effects of Malathion on Plasma ChE Activity in Male Volunteers

Time after Dosing	0.5 mg.kg ⁻¹	1.5 mg.kg ⁻¹	5.0 mg.kg ⁻¹	10.0 mg.kg ⁻¹	15.0 mg.kg ⁻¹	Placebo
Baseline	0	0	0	0	0	0
+ 1 h	+5.92*	-14.37	-5.26	-5.57	-10.07	-7.07
+2 h	+9.59	-11.62	-2.69	-4.64	-9.95	-4.34
+4 h	+14.23*	-7.83	-3.58	-3.94	+13.47*	+1.51
+8 h	-8.48	-3.45	-7.02	-7.15	-13.45	-7.89
+12 h	-11.83	-3.26	-7.63	-7.20	-10.47	-7.22
+24 h	-8.23	-6.74	-2.14	-1.49	-9.22	-5.20
+48 h	-6.79	-7.65	-3.75	-2.09	-6.33	-4.62
Day 4	-0.39	-2.99	+4.90	+0.11	-0.66	-2.22
Day 7	-13.90	-8.82	-2.83	-0.64	-1.54	-5.69
Day 14	-4.62	+0.73	-0.54	+2.48	-1.22	-3.74

Values are percent change from baseline. Positive values indicate plasma ChE activity above baseline (% increase) and negative values indicate plasma ChE below baseline (% decrease)

* Value statistically significantly different from placebo (p<0.05) after Bonferroni adjustment

This table has been derived from Table M1.1.1

TABLE 6

Effects of Malathion on Plasma ChE Activity in Female Volunteers

Time after Dosing	15.0 mg.kg ⁻¹	Placebo
*Baseline	0	0
+ 1 h	-7.68	-9.28
+2 h	-4.91	-6.76
+4 h	-4.99	-4.79
+8 h	-5.87	-3.68
+12 h	-6.65	-1.73
+24 h	-0.21	-4.21
+48 h	-2.35	-7.21
Day 4	+0.92	-2.74
Day 7	-2.68	-4.46
Day 14	+2.48	+0.70

Values are percent change from baseline. Positive values indicate plasma ChE activity above baseline (% increase) and negative values indicate plasma ChE below baseline (% decrease)

*Baseline is defined as the mean of all pre-dose assessments except screening

This table has been derived from Table M1.1.2

10.2.1 Plasma Cholinesterase

Group plasma ChE values and percentage change from baseline are summarised by gender, dose level and time after dosing in Table M2.1.

Individual subject values are shown in Table M3. Group mean results for percentage change from baseline, are shown in Tables 5 and 6 for males and females, respectively. The results are also illustrated graphically in Figures 1 and 2 (also Figures M1.1.1 and M1.2.1) for male and female subjects, respectively.

The results of the statistical analyses are summarised in Tables M1.1.1 and M1.1.2 for males and females, respectively.

Individual subject plasma ChE values are shown in Table M3 and percentage changes from baseline are illustrated in Figures M1.1.2 – M1.1.7 (males) and Figures M1.2.2 and M1.2.3 (females).

10.2.1.1 Male subjects

The data in Table 5, Table M2.1 and Figure 1 clearly show no consistent trends or relationships between group plasma ChE levels and either malathion dose or time after treatment. Table M1.1.1 shows the test for a linear trend with dose, carried out at each of ten timepoints after dosing (1 h to 14 days) and indicates significance only at 2 h and Day 7 after dosing ($p=0.016$ and $p=0.004$, respectively). The slope parameter at 2 h was negative, indicating a decrease in plasma ChE levels with increasing dose while the slope parameter at Day 7 was positive indicating an increase in plasma ChE level with increasing dose. Pairwise comparisons (treated vs placebo) at 2 h and Day 7 showed that the only significant difference occurred ($p=0.003$ at the 5% level) in the 0.5 mg.kg^{-1} group at 2 h. However, the 0.5 mg.kg^{-1} dose group had a plasma ChE level higher than that of the placebo group, a factor that undoubtedly played a role in determining the significant trend observed at 2 h. None of the pairwise comparisons between dose levels and placebo was found to be significant at Day 7.

At all timepoints other than 2 h and Day 7 the test for a linear trend with dose was not found to be significant and the Bonferroni adjustment was applied to the pairwise comparisons. The Bonferroni adjustment has been used to adjust for multiple comparisons when there is no evidence of a significant linear trend over the dose levels at each timepoint. It was decided to perform this adjustment only where there was no significant linear trend as in such circumstances spurious results would be more likely. Following the Bonferroni adjustment, statistically significant pairwise differences (5% level) were found between 15 mg.kg^{-1} and

placebo at 4 h ($p < 0.0061$), between 0.5 mg.kg^{-1} and placebo at 4 h ($p = 0.0065$) and between 0.5 mg.kg^{-1} and placebo at 1 h ($p = 0.005$). In each of these cases, however, the plasma ChE levels in the treated subjects were higher than in the subjects receiving placebo. At dose levels up to and including 15 mg.kg^{-1} , malathion did not cause any inhibition of plasma ChE in male subjects. This is shown graphically in Figure 1.

10.2.1.2 Female Subjects

The pairwise comparisons between the 15.0 mg.kg^{-1} dose and placebo were not found to be statistically significant at any time after treatment. Malathion, at 15 mg.kg^{-1} , did not cause any inhibition of plasma ChE in female subjects. The results are shown in Table 6, Tables M2.1, M1.1.2 and M3 and are illustrated graphically in Figures M1.2.1 – M1.2.3. These data are also shown graphically in Figure 2.

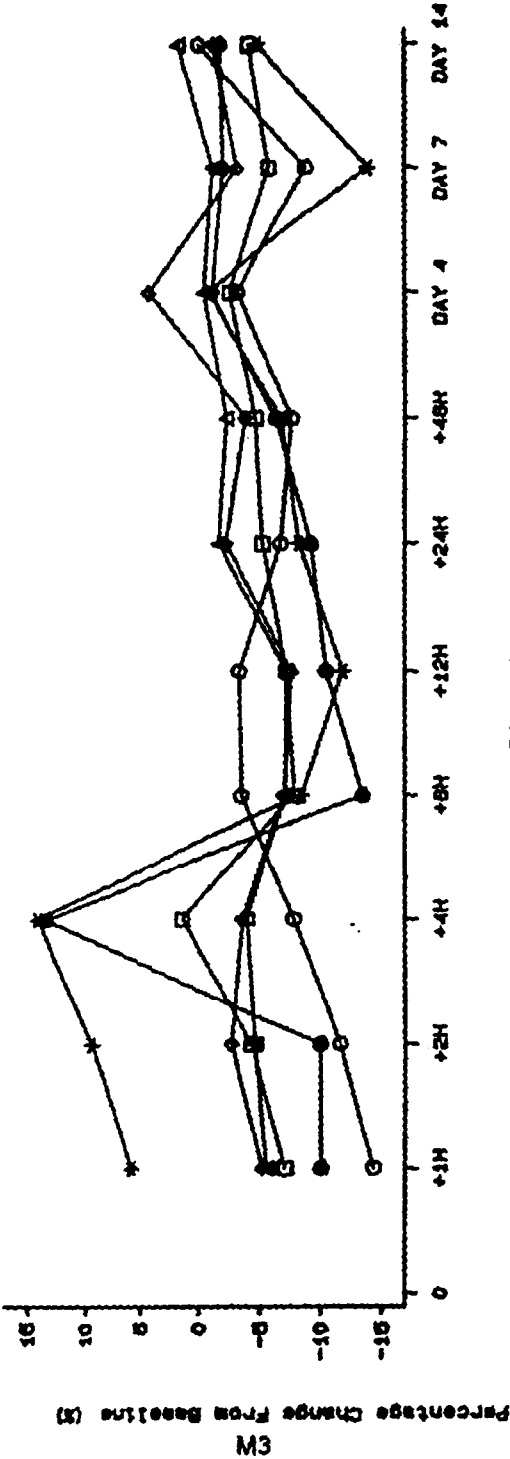
10.2.2 RBC Cholinesterase

Group RBC ChE values and percentage change from baseline are summarised by gender, dose level and time after dosing in Table M2.2. Percentage changes from baseline are also presented in Tables 7 and 8 for males and females, respectively and are illustrated graphically by gender and dose level in Figures M2.1.1 and M2.2.1 for the male and female subjects, respectively. The results of the statistical analyses for trend and pairwise comparisons are summarised in Tables M1.2.1 and M1.2.2 for males and females, respectively.

Individual subject RBC ChE values are shown in Table M3 and percent changes from baseline are illustrated graphically in Figures M2.1.2 – M2.1.7 (males) and Figures M2.2.2 and M2.2.3 (females). These data are also shown graphically in Figures 3 and 4.

FIGURE 1

Plasma Cholinesterase
Percentage Change From Baseline
Mean Values: Males



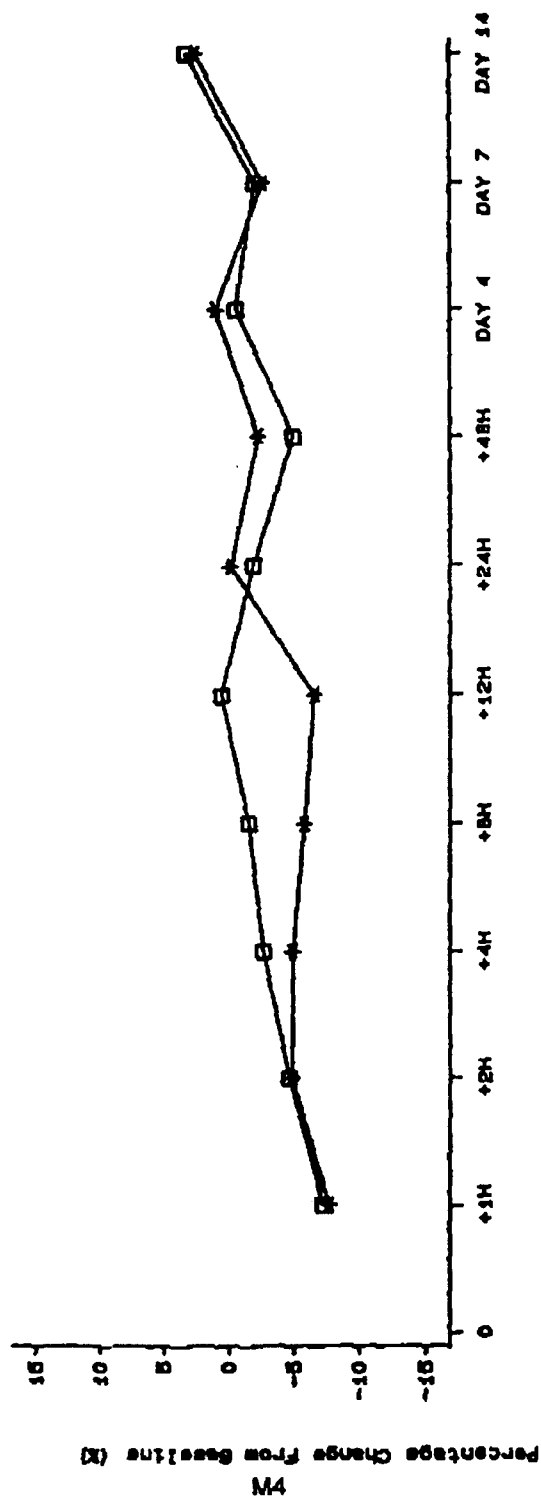
Time Point

Dose of Malathion □-□-□ PLACEBO ▲-▲-▲ 0.5 MG/KG ◆-◆-◆ 1.5 MG/KG
 ◆-◆-◆ 5.0 MG/KG ▴-▴-▴ 10.0 MG/KG ●-●-● 15.0 MG/KG

Note: The timepoints on the x-axis are not shown to scale
Baseline is defined as the mean of all predose assessments except screening
18MAY1999 10:41

FIGURE 2

Plasma Cholinesterase
Percentage Change From Baseline
Mean Values: Females



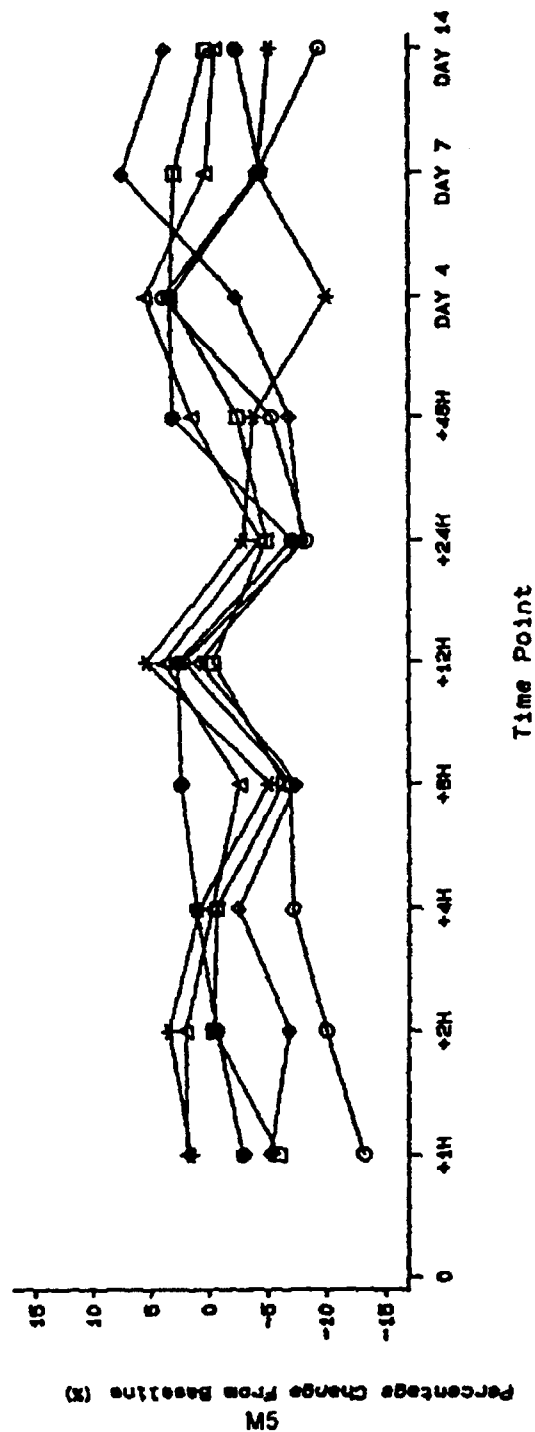
Time Point

Dose of Malathion 5-5-5 PLACEBO *** 15.0 MG/KG

Note: The timepoints on the x-axis are not shown to scale
Baseline is defined as the mean of all predose assessments except screening
18MAY1999 10:41

FIGURE 3

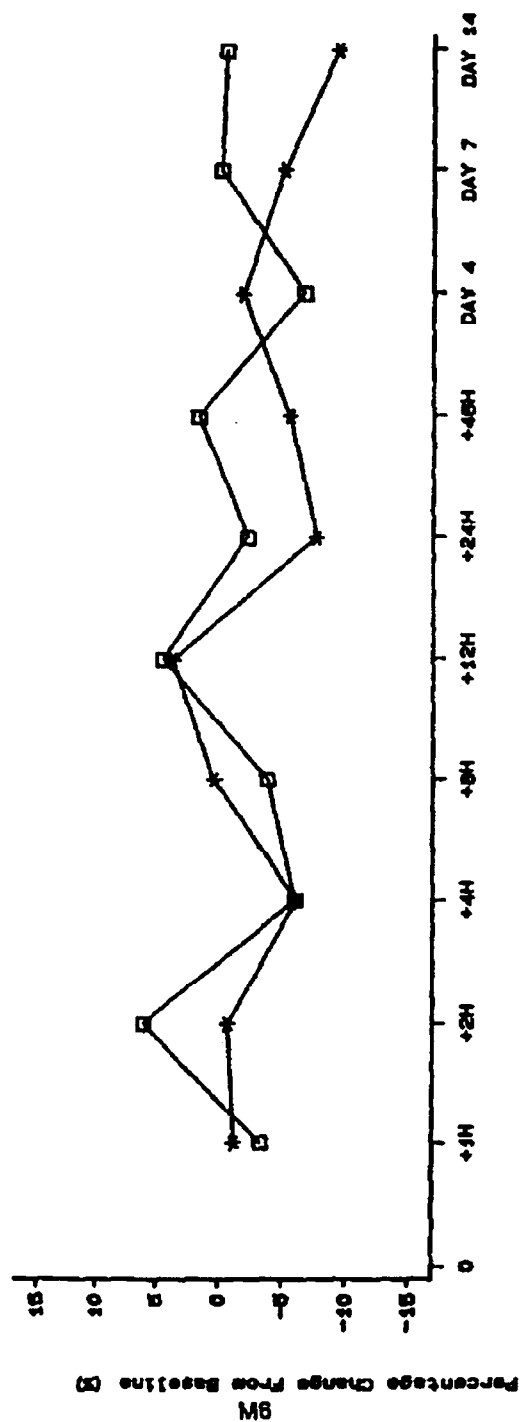
RBC Cholinesterase
Percentage Change From Baseline
Mean Values: Males



Note: The timepoints on the x-axis are not shown to scale
Baseline is defined as the mean of all predose assessments except screening
18MAY1999 10:55

FIGURE 4

RBC Cholinesterase
Percentage Change From Baseline
Mean Values: Females



Dose of Malathion 5-5-5 PLACEBO *** 15.0 MG/KG

Note: The timepoints on the x-axis are not shown to scale
Baseline is defined as the mean of all predose assessments except screening
18MAY1998 10:58

In Tables 7 and 8, positive values indicate ChE activity above baseline while negative values indicate ChE activity below baseline.

TABLE 7

Effects of Malathion on RBC ChE Levels in Male Volunteers

Time after Dosing	0.5 mg.kg ⁻¹	1.5 mg.kg ⁻¹	5.0 mg.kg ⁻¹	10.0 mg.kg ⁻¹	15.0 mg.kg ⁻¹	Placebo
*Baseline	0	0	0	0	0	0
+ 1 h	+1.55	-13.19	-5.17	+1.91	-2.86	-5.94
+2 h	+3.56	-10.04	-6.81	+2.09	-0.61	-0.35
+4 h	+0.97	-7.11	-2.43	-0.16	+1.17	-0.54
+8 h	-4.99	-6.86	-7.34	-2.55	+2.52	-6.27
+12 h	+5.54	+2.33	+0.92	+4.03	+2.84	-0.25
+24 h	-2.83	-8.24	-7.85	-4.30	-6.97	-4.78
+48 h	-3.73	-5.22	-6.75	+1.63	+3.22	-2.35
Day 4	-10.02	+4.07	-2.21	+5.64	+3.49	+3.50
Day 7	-4.08	-4.10	+7.66	+0.41	-4.35	+3.12
Day 14	-5.06	-9.30	+3.97	-0.39	-2.18	+0.44

Values are percent change from baseline. Positive values indicate RBC ChE activity above baseline (% increase) and negative values indicate RBC ChE below baseline (% decrease)

*Baseline is defined as the mean of all pre-dose assessments except screening

This table has been derived from Table M1.2.1

TABLE 8

Effects of Malathion on RBC ChE Levels in Female Volunteers

Time after Dosing	15.0 mg.kg ⁻¹	Placebo
*Baseline	0	0
+ 1 h	-1.20	-3.23
+2 h	-0.67	+6.13
+4 h	-6.15	-5.98
+8 h	+0.40	-3.75
+12 h	+3.59	+4.48
+24 h	-7.74	-2.19
+48 h	-5.63	+1.70
Day 4	-1.91	-6.70
Day 7	-5.25	-0.10
Day 14	-9.45	-0.51

Values are percent change from baseline. Positive values indicate RBC ChE activity above baseline (% increase) and negative values indicate RBC ChE below baseline (% decrease)

*Baseline is defined as the mean of all pre-dose assessments except screening

This table has been derived from Table M1.2.2

10.2.2.1 Male Subjects

The test for a linear trend with dose at each timepoint was not found to be significant at any time after treatment.

Consequently, the Bonferroni adjustment was applied to the pairwise comparisons between all dose levels and placebo. None of the pairwise comparisons between dose groups and placebo were found to be statistically significant at the 5% level. At dose levels up to and including 15 mg.kg^{-1} , malathion did not cause any inhibition of RBC ChE in male subjects. This is shown graphically in Figure 3.

10.2.2.2 Female Subjects

The pairwise comparisons between the 15.0 mg.kg^{-1} dose and placebo were not found to be statistically significant at any time after treatment. Malathion, at 15 mg.kg^{-1} , did not cause any inhibition of RBC ChE in female subjects. This is shown graphically in Figure 4.

10.2.3 Statistical/Analytical Issues

Normality and homogeneity of variance are assumed for pairwise comparisons.

These assumptions were considered to be reasonably satisfied for both response variables for the statistical analysis of the female subjects and for the statistical analysis of the percentage change from baseline in RBC ChE for the male subjects. There was some doubt over the validity of the assumptions for the statistical analysis of the percentage change from baseline in plasma ChE for the male subjects and the plots of the residuals showed that this was possibly due to a number of outliers.

Outliers were defined as values less than the lower quartile value minus one and a half times the interquartile range or values greater than the upper quartile value plus one and a half times the interquartile range. Using the above definition, 9 outliers were obtained. All 7 subjects receiving the 15.0 mg/kg dose level at the 4 h timepoint, and subjects 025 and 032 on placebo at the 12 h and 4 h timepoints respectively, had

values that were found to be outliers. As a result, the 15.0 mg/kg dose level would be required to be excluded from any statistical analysis at the 4 h timepoint. The statistical methods in the protocol would, therefore, not be appropriate and alternative statistical techniques would be required. However, when the residual values were compared for the outliers it was found that there were 4 negative values and 5 positive values. As there were similar numbers of outliers in each direction and these were of similar magnitude, they should offset each other, and the results should not be altered significantly; it was therefore considered appropriate not to perform any statistical analysis on the reduced dataset and to report only the results of the full dataset.

For the male subjects there was generally no indication of a quadratic relationship of plasma and RBC ChE with dose level; for female subjects this was not investigated since only one dose level of malathion was administered. With the analysis methods used there was no easy way to test for deviations from linearity. Consequentially instead of performing such a test we tested for a quadratic dosage effect and determined whether there was a significant quadratic trend (deviations from linearity) prior to conducting the linear trend test. There were no significant quadratic trends so the linear trend test results were reported.

10.2.4 Conclusion of Effects on Blood ChE

For male subjects a significant dose-related trend in plasma ChE levels was observed at 2 h and Day 7 after treatment. The trend at 2 h indicated a decreasing level of plasma ChE with increasing dose while the trend at Day 7 indicated the opposite, an increasing level of plasma ChE with increasing dose. The positive dose-related trend observed at 2 h was not supported by pairwise comparisons between plasma ChE levels in treated subjects and placebos. In fact, these comparisons showed that the only significant pairwise difference at 2 h was between the 0.5 mg.kg⁻¹ dose level and placebo and indicated that plasma ChE levels in the treated subjects were higher than in the subjects receiving placebo. Similar pairwise comparisons between all dosed groups and

placebos at all other times after treatment indicated significant differences only in the 0.5 mg.kg⁻¹ group at the 1 h timepoint and in the 0.5 mg.kg⁻¹ and 15 mg.kg⁻¹ groups at 4 h after treatment. All of these differences indicated plasma ChE levels in treated groups to be higher than that in subjects receiving placebo. Together, these results do not suggest any effect of malathion on plasma ChE in male subjects at any dose level tested.

For female subjects, pairwise comparisons of plasma ChE between the 15 mg.kg⁻¹ dose group and the placebo group were not found to be statistically significant at any time after treatment.

It is concluded that, at dose levels up to and including 15 mg.kg⁻¹, malathion did not cause any inhibition of plasma ChE in either male or female subjects.

For male subjects, there were no dose-related trends in levels of RBC ChE and no significant change in RBC ChE levels (relative to baseline) at any timepoint after treatment. No statistically significant pairwise differences in RBC ChE were found between any of the dose levels and the placebo.

For female subjects, pairwise comparisons of RBC ChE levels between the 15 mg.kg⁻¹ dose group and placebo were not found to be statistically significant at any time after treatment.

It is concluded that, at dose levels up to and including 15 mg.kg⁻¹, malathion did not cause any inhibition of RBC ChE in either male or female subjects.

10.3 ADVERSE EVENTS (AES)

All adverse events, defined as any unwanted events occurring during the course of the study, are summarised in Table 9 and Table Q1.1 and those experienced by individual subjects are shown in Table Q2.1.1. Details regarding severity and possible relationship to test compound are shown in Tables Q1.2 and Q1.3, respectively.

A total of 44 adverse events were reported in 20 of 48 subjects who entered the study. This included Subject 005 (receiving 1.5 mg.kg⁻¹), Subject 014 (receiving 50 mg.kg⁻¹) and Subject 045 (receiving placebo) who had symptoms present prior to dosing (Table 9). A total of 40 adverse events after dosing were reported in 18 of 48 subjects who entered the study. Five of the 18 subjects (28%) received placebo and 13 (72%) received test compound. These percentages with adverse events after dosing are almost identical to the percentages of the subjects receiving placebo and test compound in this study (29% and 71% respectively). In males, adverse events were reported in 3 of 11 (27%) subjects receiving placebo, one of 3 (33%) subjects receiving 0.5 mg.kg⁻¹, 2 of 3 (67%) receiving 1.5 mg.kg⁻¹, 3 of 7 (43%) subjects receiving 5 mg.kg⁻¹, 2 of 7 (29%) subjects receiving 10 mg.kg⁻¹ and 2 of 7 subjects (29%) receiving 15 mg.kg⁻¹ of malathion. In female subjects, adverse events were reported in 2 of 3 (67%) subjects receiving placebo and 3 of 7 (43%) receiving 15 mg.kg⁻¹ malathion (Table Q1.1).

None of the recorded adverse events exceeded a severity of 2 on a scale of 1-4 with the exception of a pre-existing perianal abscess (Subject 014) that subsequently required hospitalisation (no relationship to test compound).

The possible relationship of the reported adverse events to the test compound is indicated in Tables Q1.3 and Q2.1.2. It should be noted, however, that initial conclusions regarding possible test compound-relatedness were made by the investigator when the study was blinded and when no information was available regarding levels of ChE inhibition or urinary elimination of the test compound. Final conclusions regarding possible relationships between adverse events and the test compound took several factors into consideration, such as placebo vs test compound, clinical and other effects observed and time after treatment. A summary of the final conclusions regarding the occurrence of adverse effects is shown in Table 9. This indicates that there is no increase in the incidence of reported adverse effect with dose level and that, in the absence of any evidence of inhibition of plasma or RBC ChE, any association with the test compound is unlikely.

TABLE 9

Adverse Events

Subject No.	Dosing Level mg.kg ⁻¹	Symptoms	Severity	Time After Dosing	ChE Inhibition Y/N	Treatment Relationship Y/N	
						Blinded	Unblinded
002	0.5	Headache	Grade 1	2 Days 8 h 24 min	N	Unlikely	N
		Rhinitis	Grade 1	11 Days 23 h 54 min	N	N	N
005	1.5	Dizziness	Grade 1	Present prior to dosing	N	N	N
006	1.5	Rhinitis	Grade 2	5 Days 10 h 50 min	N	N	N
		Conjunctival haemorrhage	Grade 1	5 Days 23 h 35 min	N	N	N
008	1.5	Headache	Grade 1	6 h 10 min	N	Possibly	N
		Skin reaction, localised	Grade 1	14 h 10 min	N	N	N
		Elevated aspartate aminotransferase	Grade 1	1 Day	N	Possibly	N
		Elevated alanine aminotransferase	Grade 1	1 Day	N	Possibly	N
		Pharyngitis	Grade 1	4 Days 2 h 55 min	N	N	N
010	5.0	Headache	Grade 2	2 h 50 min	N	Possibly	N
		Nausea	Grade 1	7 h 5 min	N	Possibly	N
011	5.0	Flatulence	Grade 1	4 h 15 min	N	Possibly	N
012	5.0	Rhinitis	Grade 1	7 Days 22 h 40 min	N	N	N
013	placebo	Flatulence	Grade 2	21 h 34 min	N	Unlikely	N
014	5.0	Perineal pain	Grade 1	present prior to dosing	N	N	N
		Perianal abscess	Grade 3	present prior to dosing	N	N	N
019	10.0	Headache	Grade 2	1 h	N	Possibly	N
		Headache	Grade 1	8 h 5 min	N	Possibly	N
		Headache	Grade 1	2 Days 2 h 15 min	N	Unlikely	N
		Headache	Grade 2	3 Days 7 h	N	Unlikely	N
021	placebo	Headache	Grade 1	3 h 20 min	N	Possibly	N
		Headache	Grade 1	1 h	N	Possibly	N
022	10.0	Fever	Grade 1	8 h 3 min	N	N	N
		Sweating increased	Grade 1	8 h 5 min	N	Unlikely	N
		Dizziness	Grade 1	8 h 45 min	N	Possibly	N
		Flatulence	Grade 1	10 h 45 min	N	Possibly	N
		Headache	Grade 2	5 Days 6 h 30 min	N	N	N
		Headache	Grade 2	5 Days 6 h 30 min	N	N	N
034	15.0	Dizziness	Grade 1	1 h 59 min	N	Possibly	N
035	placebo	Abdominal pain	Grade 1	2 Days 1 h 15 min	N	N	N
037	15.0	Headache	Grade 1	2 Days 5 min	N	N	N
		Nausea	Grade 1	2 Days 5 min	N	N	N
040	15.0	Skin dry	Grade 1	12 Days 36 min	N	N	N
041	placebo	Eye pain	Grade 1	2 Days 8 h 30 min	N	N	N

TABLE 9 (continued)

Adverse Events

Subject No.	Dosing Level mg.kg ⁻¹	Symptoms	Severity	Time After Dosing	ChE Inhibition Y/N	Treatment Relationship Y/N	
						Blinded	Unblinded
042	15.0	Headache	Grade 1	2 Days 5 h 25 min	N	Unlikely	N
		Sweating increased	Grade 1	5 Days 14 min 10 min	N	N	N
		Headache	Grade 1	5 Days 14 h 10 min	N	N	N
		Paraesthesia	Grade 1	5 Days 14 h 10 min	N	N	N
		Tooth ache	Grade 1	8 Days 23 h 25 min	N	N	N
045	Placebo	Headache	Grade 1	8 h 3 min	N	Possibly	N
		Abdominal cramp	Grade 1	present prior to dosing	N	N	N
		Fever	Grade 1	3 h 56 min	N	Unlikely	N
		Tooth ache	Grade 1	5 Days 9 h 38 min	N	N	N
046	15.0	Headache	Grade 2	30 min	N	Possibly	N

10.3.1 Analysis of Adverse Events that Occurred During the Study**Males (Placebo)**

A total of 3 adverse events were reported in the male placebo group.

Subject 013 reported a brief period (5 min) of flatulence 21.5 h after dosing. This was initially reported when blinded as 'unlikely' related to the test compound. The final conclusion is that it is 'unrelated' to treatment.

Subject 021 experienced a headache starting a little over 3 h after dosing and lasting for about one day. Prior to unblinding this was reported as 'possibly' related to the test compound. The final conclusion is that it is 'unrelated' to treatment.

Subject 035 experienced abdominal pain (cramping) beginning 2 days after dosing. This was considered 'unrelated' to the test compound.

Males (0.5 mg.kg⁻¹ Malathion)

One subject (002) experienced a headache starting 2.5 days after treatment and rhinitis starting 12 days after treatment. When blinded, the investigator concluded these events were 'unlikely' to be related and 'not related' to treatment, respectively. Since the headache did not begin until 2 days after treatment and no inhibition of ChE was evident, it is concluded that neither event is related to treatment.

Males (1.5 mg.kg⁻¹ Malathion)

Three adverse events were reported in this group.

Subject 005 experienced dizziness for 4 min following blood sampling prior to dosing. This was 'unrelated' to the test compound.

Subject 006 experienced rhinitis and a subconjunctival haemorrhage 5 days after dosing. These events were both linked to a viral infection and 'unrelated' to the test compound.

Subject 008 experienced headache, red spot (on ring finger), hepatic function abnormalities (raised aspartate aminotransferase and alanine aminotransferase) and pharyngitis. The skin reaction and pharyngitis were considered 'unrelated' to the test compound. When the study was blinded, the headache and hepatic disturbance starting about 6 h and 1 day after dosing, respectively, were considered 'possibly' related to the test compound. However, since no inhibition of either plasma or RBC ChE was observed concurrent with these events, test compound relatedness is considered 'unlikely'. Subject 008 had recently had a cold (with headache and cough) prior to starting the study.

5 mg.kg⁻¹ Malathion (Males)

Four subjects in this group (010, 011, 012 and 014) experienced adverse events.

Subject 010 experienced headache and nausea beginning about 3 h and 7 h after dosing respectively which resolved after about 20 h and 11 h respectively. These events were considered 'possibly' related to the test compound when the study was blinded. Since there was no evidence of any inhibition of plasma or RBC ChE during the period when the events were experienced, it is 'unlikely' that they were related to the test compound.

Subject 011 experienced flatulence starting about 4 h after dosing and lasting for over one day. Prior to unblinding this event was considered 'possibly' related to the test compound. Since there was no evidence of any inhibition of plasma or RBC ChE during the period when the events were experienced, it is 'unlikely' that they were related to the test compound.

Subject 012 experienced rhinitis starting about 8 days after dosing. This was considered 'unrelated' to the test compound.

Subject 014 experienced pre-dose pain in the perineum that was subsequently diagnosed as a perianal abscess. Although this was recorded as a serious adverse event and required hospitalisation, this event was unrelated to the test compound.

Males (10 mg.kg⁻¹ Malathion)

Two subjects in this group (019, 022) experienced adverse events.

Subject 019 experienced an essentially continuous headache during the first 6 days after dosing. Before unblinding, the headache was reported as 'possibly' related to the test compound during the first day after treatment but 'unlikely' to be related after a few days. Since there was no evidence of any inhibition of plasma or RBC ChE during the period when the headache occurred, it is 'unlikely' that it was related to the test compound.

Subject 022 experienced a headache at 1 h and 5 days after treatment and reported other events (fever, sweating, dizziness and flatulence) within the first day after dosing. When the study was blinded, the initial headache, dizziness and flatulence were considered 'possibly' related to the test compound. However, there was no evidence of any inhibition of plasma or RBC ChE during the period when these events occurred and it is 'unlikely' that they were related to test compound.

Males (15 mg.kg⁻¹ Malathion)

Two subjects (034 and 037) in this group reported adverse events.

Subject 034 experienced a very brief (1 min) period of dizziness approximately 2 h after dosing that was considered 'possibly' related to the test compound when the study was blinded. However, there was no evidence of any inhibition of plasma or RBC ChE during the period when this occurred and it is 'unlikely' that it was related to the test compound.

Subject 037 experienced a headache and nausea but these did not start until 2 days after treatment. As a result, they were considered 'unrelated' to the test compound.

Females (Placebo)

Two subjects (041 and 045) experienced adverse events.

Subject 041 experienced eye pain starting 2 days after treatment that was considered 'unrelated' to the test compound.

Subject 045 experienced headache starting about 8 h after dosing that was initially considered 'possibly' related to test compound prior to unblinding. Since subject 045 received placebo the effect is 'unrelated' to the test compound as were cramps experienced during the first day of dosing and fever and toothache reported subsequently.

Females (15 mg.kg⁻¹)

Three subjects in this group (040, 042 and 046) experienced adverse events.

Subject 040 experienced dry skin under her eye starting 12 days after treatment. This effect was considered 'unrelated' to test compound.

Subject 042 experienced headache on two occasions, sweating, paresthesia and toothache. However, none of these events occurred until two days after treatment, were not associated with any inhibition of blood ChE and were considered to be 'unrelated' to the test compound.

Subject 046 experienced a headache starting about 30 min after dosing. When the study was blinded this was considered 'possibly' related to the test compound. However, there was no evidence of any inhibition of plasma or RBC ChE during the period when this event occurred and it is 'unlikely' that it was related to the test compound.

10.3.2 Conclusion on Adverse Effects

A total of 40 adverse events were reported in 18 subjects following administration of malathion or placebo.

A total of 5 out of 14 (36%) subjects receiving placebo experienced at least one adverse event during the study compared with 13 out of 34 (38%) treated subjects. Consequently, the incidence of adverse events appears to be uniformly distributed among subjects receiving test compound and placebo.

With the exception of the pre-existing perianal abscess in Subject 014, all of the adverse events experienced were relatively mild and did not exceed Grade 2 in severity. In the few cases where the initial "blinded" conclusion suggested a 'possible' relationship to treatment, the absence of any evidence of inhibition of plasma or RBC ChE, makes any association with the test compound unlikely.

Among treated subjects, there was no evidence that there was an increased incidence of adverse events with increasing dose level.

Typical historical control data of adverse events in healthy volunteers from some studies conducted at ICR are included in Appendix T.

10.3.3 Serious Adverse Event

No subject was withdrawn from the study as a result of an adverse event.

There was one serious adverse event reported in Subject 014. This was a perianal abscess which required the subject to be hospitalised. This event was not related to the test compound.

10.4 CLINICAL LABORATORY VITAL SIGNS EVALUATION

Vital signs

Summary data for vital signs are provided in Tables I1.1 – I1.6. Individual values are provided in Table I2.

Vital sign measurements showed minor increases and decreases from baseline values (supine systolic blood pressure, diastolic blood pressure, supine pulse rate, or erect heart rate). These changes were also observed in subjects receiving placebo capsules and were not clinically significant in any subject during the course of the study.

Electrocardiograph (ECG)

Summary data for ECG values are provided in Tables J1.1 – J1.5. Individual values are provided in Tables J2.1 and J2.2.

There were no clinically significant abnormalities or changes noted in any 12-lead ECG for any subject during the study.

Laboratory Investigations

Haematology

Summary data for haematology results are provided in Tables K1.1 – K1.14. Individual values are provided in Table K2.1.

Minor differences from normal range values were noted for several haematological parameters-haemoglobin, red blood count, haematocrit, mean cell haemoglobin, mean cell volume, mean cell haemoglobin concentration, and/or white cell count. These changes were considered not to be treatment related because they were either increases or decreases and were not considered to be clinically significant. In addition, there was no dose-response and several of these changes were also present in subjects receiving placebo capsules.

Clinical Chemistry

Summary data for clinical chemistry results are provided in Tables L1.1 – L1.13. Individual values are provided in Table L2.1.

Minor differences from normal range values were noted for several clinical chemistry parameters – glucose, AST, ALT, LDH, Na, K, Cl, TP, Alb, and/or T. bil. These changes were not considered to be treatment related because they were either increases or decreases and were not considered to be clinically significant. In addition, several of these changes were also present in subjects receiving placebo capsules.

Subject 008 (1.5 mg.kg⁻¹) had an increased AST and ALT values, 78 iu.l⁻¹ and 102 iu.l⁻¹, respectively, 24 h after dosing. Although these results were recorded as adverse events they are not indicative of treatment relatedness because they occurred in one subject at the low dose level only and the results returned to normal 5 days later (AST 16 iu.l⁻¹ and ALT 36 iu.l⁻¹). In addition, there was no inhibition of either blood plasma or RBC ChE activity for this subject at this timepoint.

Urinalysis

Summary data for urinalysis results are provided in Tables N1.1 and N1.2. Individual values are provided in Table N2.1 and N2.2.

For most subjects urinalysis values were normal. Occasionally trace amounts of protein, blood, ketones, or glucose were seen. Microscopic examination of sediments for these urine samples showed corresponding presence of either red blood cells (0-2 per HPF), white blood cells (0-8 per HPF), or casts (0-6 per HPF). Most of these abnormalities were present in urine samples analysed on screening day, and were also present in subjects receiving placebo capsules. None of these abnormalities were considered to be of clinical significance and none were considered to be related to the test compound.

Physical Examination

Individual values are given in Tables H2.4.1 to H2.4.4

There was no change from baseline in physical examination for any subject.

Conclusions

There were no clinically significant changes in vital signs, ECGs, haematology, clinical chemistry, urinalysis or physical examination in any subject during the study.

10.5 PHARMACOKINETIC RESULTS

10.5.1 Plasma Analysis

Since no inhibition of either plasma or RBC was observed at any dose level employed, it was decided that, initially, analysis of plasma for malathion and malaoxon would be restricted to samples from volunteers who were dosed at 15 mg.kg⁻¹ and the corresponding placebos. As a consequence, plasma samples from 7 male and 7 female volunteers who had each received 15 mg.kg⁻¹ malathion and a total of 8 placebos (5 male and 3 female) were analysed for

malathion and malaoxon by Analytical Method No. 6674 (see Inveresk Report No. 17123 of Inveresk Project No. 366748). In all cases, plasma samples collected pre-dose and at 1, 2, 4, 8 and 12 h post-dose were analysed.

The results, shown in Appendix R (Tables 1-3), clearly show that the plasma concentrations of both malathion and malaoxon were below quantifiable levels 1-12 h after dosing in all male and female volunteers who received 15 mg.kg⁻¹ malathion or placebo. Based on the results of the method validation, plasma levels of malathion were <ca 102 ng.ml⁻¹ plasma and of malaoxon were <ca 99.8 ng.ml⁻¹ plasma in volunteers dosed at 15 mg.kg⁻¹ malathion. Since this was the highest dose of malathion employed, plasma samples of volunteers receiving lower doses were not analysed.

Analytical data for plasma concentrations of malathion and malaoxon are presented in Appendix R.

11. OVERALL CONCLUSION

A total of 40 adverse events were reported in 18 subjects following the administration of malathion or placebo. The incidence of adverse events appeared to be uniformly distributed among subjects receiving test compound and placebo. Among treated subjects, there was no evidence that there was an increased incidence of adverse events with increasing dose level.

There were no clinically significant changes in vital signs, ECGs, haematology, clinical chemistry, urinalysis or physical examination in any subject during the study.

At dose levels up to and including 15 mg.kg⁻¹, malathion did not cause any inhibition of plasma or RBC ChE in either male or female subjects.

Plasma concentrations of both malathion and malaoxon were below quantifiable levels 1-12 h after dosing in all male and female volunteers who received 15 mg.kg⁻¹ malathion or placebo. Based on the results of the method validation, plasma levels of malathion were <ca 102 ng.ml⁻¹ plasma and of malaoxon were <ca 99.8 ng.ml⁻¹ plasma in volunteers dosed

at 15 mg.kg⁻¹ malathion. Since this was the highest dose of malathion employed, plasma samples of volunteers receiving lower doses were not analysed.

Malathion was tolerated well at doses ranging from 0.5 mg.kg⁻¹ to 15 mg.kg⁻¹ in male subjects and at a dose of 15 mg.kg⁻¹ in female subjects.

It is concluded that a minimum No Observed Effect Level of malathion when given as a single oral dose to healthy male and female human volunteers is 15 mg.kg⁻¹.

APPENDIX A

Protocol and amendments, blank case record form

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ICR STUDY NO: 013177

**A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.**

PROTOCOL STATUS: FINAL - 19 OCTOBER 1998

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ICR 013177 - FINAL - 19 OCTOBER 1998

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Emergency Number

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Persons authorised to sign protocol and amendments

Sponsor:	Dr C F Wilkinson
ICR:	Dr S Freestone
	Dr J Dickson

I acknowledge possession of, and have read, a written summary of the pre-clinical and clinical data available on the compound to be studied in this protocol. I have had the opportunity to discuss the data and their implications with staff of the manufacturer. Having fully considered all the information available, I consider it is safe and ethically justifiable to give the compound to volunteers according to the agreed protocol.

Signed S. Jreestone Date: 20 October 1998

Study Director

SIGNATURE PAGE

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.

THE UNDERSIGNED AGREE TO PERFORM THE STUDY ACCORDING TO THIS
PROTOCOL.

Study Director: S. J. Freestone Date: 20 Oct 98

Clinical Investigator: S. J. Freestone Date: 20 Oct 98

Sponsor Representative: C. F. Williams Date: June 3, 1999

PROTOCOL CHANGES REQUESTED BY ETHICS COMMITTEE

Comments raised by the Ethics Committee were addressed in a letter by Dr S Freestone to the Chairman of the committee.

ADDITIONAL PROTOCOL CHANGES

Protocol

Page 2 of 69: Project Clinical Research Associate has been amended to Kay Whalley.

Page 3 of 69: **Sponsor Medical Contact:** Dr Anna-Mette Nielsen

Page 10 of 69: Urinary Pregnancy Test added to schedule of events in the safety section and the planned study dates have been amended.

Page 11 of 69: "in pharmacokinetic studies using radio-labelled malathion most...." Was added to the 3rd sentence in the second paragraph.

Page 12 of 69: **5th paragraph - Human Data:** The paragraph now reads "A repeat dose oral study in volunteers (24 mg per person per day for 56 consecutive days) showed an equivocal reduction in plasma and RBC Cholinesterase after cessation of dosing. The study was of dubious quality and the effect observed probably artefactual."

Page 12 of 69: **6th paragraph:** Inserted into the first sentence after 'figure' - (considered a safe daily human intake over a lifetime).

Page 13 of 69: **First sentence:** 'Major' amended to 'measurable', and 'and are very much lower than doses expected to cause any clinical signs' has been added to the end of the sentence.

Page 13 of 69: **Section 4.1.1. Second sentence:** The word 'studies' amended to 'studied'.

Page 14 of 69, 7th paragraph: The paragraph has been amended to read "In the final session, 7 females will receive the NOEL dose identified in males and 3 females will receive placebo. If a NOEL in males is not identified at doses up to and including 15 mg.kg⁻¹, further cohorts of men may be studied and dosing in females delayed until a NOEL is identified.

Page 16 of 69, Section 4.3.1, Pre-study screen, Point 2: Inserted after 'pulse rate' '(supine and erect)' and at the end of the sentence 'and oral temperature'. Point 7 has been added.

Page 17 of 69, Section 4.3.3, Exclusion Criteria: Point K amended to read "Smokers who cannot abstain from smoking from 2 hours predose to 8 hours postdose".

Page 18 of 69, Section 4.3.5, Second sentence: 'should' amended to 'will'.

Page 18 of 69: Last sentence of 5th paragraph now reads "where subjects are withdrawn because of reasons 2 or 3 they will be replaced, if insufficient data is obtained".

Page 21 of 69, Section 4.5.1.1: Last sentence has been added to paragraph 1 and the 3rd paragraph deleted.

Page 26 of 69, Section 4.5.1.5.5: First sentence has been changed to "available for use by medical staff" as 2-PAM will be kept at Edinburgh Royal Infirmary and not within the clinical unit.

Page 27 of 69, Section 4.6, 4th paragraph: Last sentence in brackets has been deleted.

Page 28 and 29 of 69, Dr M McGuire amended to D.L. Scott

Page 28 of 69, Section 4.7.2: The specific alkyl phosphates have been added to the 6th paragraph dimethyl phosphate, dimethyl thiophosphate and dimethyl dithiophosphate. The urine volume to be collected has been increased to 2x20ml aliquots.

Page 30 of 69: BP and HR amended to Vital Signs and Temperature deleted.

Page 31 of 69, Section 4.8.3, Study Day: Sentence has been added "Subjects must refrain from smoking from 2 hours predose". Paragraph 4 "Subjects in the habit of smoking may do so after this timepoint" added to the end.

Page 37 of 69, Second and third paragraph - first sentence: '-30min' has been deleted after 'ie'.

Page 44 of 69, Section 11: Dates of August and October amended to October 1998 and February 1999 respectively.

Volunteer Information

Page 51 of 69, Point 12: Blood volume has been amended to 235 mls from 180 mls.

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SUMMARY

ICR STUDY NUMBER/SPONSOR CODE	013177
TITLE:	A randomised double blind ascending single oral dose study with Malathion to determine the No Effect Level on plasma and RBC cholinesterase activity.
SPONSOR:	Cheminova Agro A/S
REGULATORY STATUS:	For EPA Submission
STUDY OBJECTIVES:	To determine the safety of Malathion and to establish a no adverse effect level in healthy human male and female volunteers.
STUDY DESIGN:	Double blind, randomised, placebo controlled.
STUDY POPULATION: Number of volunteers:	48 (4 subjects per dose level (3 active and 1 placebo) for the first two dose levels and 10 subjects per dose level thereafter (7 active and 3 placebo)).
Age/Sex: Study specific requirements:	18-50y (38 male and 10 female). Healthy, weight 50-100kg within \pm 15% ideal body weight.
INVESTIGATIONAL PRODUCT: Formulation:	Malathion
Route of administration: Dosage(s) per day:	Oral Single dose, ascending doses of 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg ⁻¹ . Females studied at NOEL dose.
EVALUATION CRITERIA: Safety:	<p>Plasma and RBC Cholinesterase: Screening, on Days -9, -7, -5, -2, -1, and -30 min predose. 1, 2, 4, 8, 12, 24 and 48h postdose and Days 4, 7 and 14.</p> <p>Vital Signs: Screening, Day-1, predose and 2h, 4h, 8h and 24h postdose.</p> <p>12 Lead ECG: Screening, -30min predose, 2h, 4h, 8h and 24h postdose.</p> <p>Continuous single channel ECG monitoring: -30min predose to 4h postdose.</p> <p>Haematology/Clinical Chemistry: Screening, predose (0) and 24h postdose.</p> <p>Urinalysis: Screening and 24h postdose.</p> <p>Urine pregnancy test: Screening and Day -1.</p> <p>Physical Examination: Screening and 48h postdose.</p> <p>Adverse Events/Clinical Signs: Throughout study.</p> <p>Urine Drug Screen: Screening.</p> <p>Virology: Screening.</p>
Pharmacokinetics:	<p>Plasma: Predose (0), 1, 2, 4, 8, 12, 24, 48 and 72h postdose</p> <p>Urine: Predose (-12-0h), 0-12h, 12-24h, 24-48h postdose</p>
STUDY LOCATION:	Inveresk Clinical Research
Clinical phase:	Inveresk Research
Clinical pathology:	Inveresk Research
Bioanalytical analysis:	Inveresk Research
Statistical analysis:	Inveresk Research
PLANNED STUDY DATES:	
Start of clinical phase:	October 1998
Completion of clinical phase:	February 1999
DURATION OF STUDY: (per subject)	14 days postdose

2. **BACKGROUND INFORMATION**

INTRODUCTION

Cheminova Agro A/S wishes to carry out an oral dosing study on malathion, an organophosphate insecticide that has been used for many years on a wide range of plants and crops in many parts of the world. It is also a constituent of lotions and shampoos used in the treatment of scabies and head lice. As a result of this use, accidental and incidental exposure of workers occur continually and the public may be exposed to residues of malathion in food, water and from residential and/or garden uses. The primary objective of the proposed study is to determine the highest dose of malathion causing no effect or lowest dose causing a slight inhibitory effect on blood cholinesterase in humans. This information will be used to provide a more accurate assessment of the margin of safety associated with currently estimated human exposures. The proposed study will investigate, under carefully controlled conditions, the effects of single oral doses of malathion on humans and will identify a dose level with no effect on plasma and RBC cholinesterase activity.

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION AND KINETICS

Malathion is rapidly and almost completely absorbed orally. Excretion is mainly in the urine, 80% as it's monocarboxylic and dicarboxylic acids which are inactive. In pharmacokinetic studies using radio-labelled malathion, most radio-label is removed from the body in 24h so there is little likelihood of accumulation of malathion or its metabolites.

TOXICOLOGY

The single dose toxicity of malathion is relatively low, with oral LD₅₀ values of at least 1000 mg.kg⁻¹. Its effects are due to inhibition of cholinesterase activity.

A 28 day dietary rat study indicated a No Observed Effect Level (NOEL) of 25 mg.kg⁻¹ based upon cholinesterase depression, and 28 day dog studies suggested cholinesterase depression at 125 mg.kg⁻¹.

A NOEL of approximately 5 mg.kg⁻¹ for cholinesterase inhibition was indicated in a chronic rat dietary study.

GENOTOXICITY AND CARCINOGENICITY

Ames tests were negative. Positive in-vivo chromosomal aberration results occurred in mice and hamsters but not rats.

A series of carcinogenicity studies indicated no clear carcinogenic activity. Any such potential seems to be slight and of little relevance to a single dose study.

HUMAN DATA

A repeat dose oral study in volunteers (24 mg per person per day for 56 consecutive days) showed an equivocal reduction in plasma and RBC Cholinesterase after cessation of dosing. The study was of dubious quality and the effect observed probably artifactual.

The Acceptable Daily Intake (ADI) figure (considered a safe daily human intake over a lifetime) suggested by the Pesticide Safety Division is 0.05 mg.kg⁻¹ day⁻¹ calculated using a 100-fold safety factor in the NOEL in a rat carcinogenicity study. The JMPR summary suggested 0.3 mg.kg⁻¹ as an ADI for man.

OVERVIEW

Malathion is rapidly absorbed after oral administration and almost entirely excreted within 24 hours.

It is of moderate to low toxicity with oral LD₅₀ values of 1000 mg.kg⁻¹ and greater. The main effect is inhibition of cholinesterase.

The proposed doses of 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹ are unlikely to have measurable effects on cholinesterase activity, and are very much lower than doses expected to cause any clinical signs.

3. STUDY OBJECTIVES

The study is to be undertaken in male human volunteers to establish an acute, single dose, oral No-Observed-Effect-Level (NOEL). This is defined as the highest dose tested at which no inhibition of plasma and red blood cell cholinesterase activity occurs. A group of female volunteers will be studied subsequently at the NOEL identified in males.

4. METHODS AND INVESTIGATIONAL PLAN

4.1 Study design and plan

4.1.1 Design of Study

Dose ranging covers 5 doses (0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹) and involves a maximum of 48 subjects (Table 1). If these doses do not result in inhibition of cholinesterase activity, higher doses may be studied after consultation with the Ethics Committee and issue of an amendment. The study is a double blind comparison of the test compound against placebo.

TABLE 1

	Placebo	0.5 mg.kg ⁻¹	1.5 mg.kg ⁻¹	5.0 mg.kg ⁻¹	10.0 mg.kg ⁻¹	15.0 mg.kg ⁻¹	NOEL dose in %
Session 1 Sub 001-004	1	3					
Session 2 Sub 005-008	1		3				
Session 3 Sub 009-018	3			7			
Session 4 Sub 019-022	1				3		
Session 5 Sub 023-031	2				4	3	
Session 6 Sub 032-038	3					4	
Session 7 (A) Sub 039-048	3						7

The study will be executed in 7 treatment blocks. The first block will comprise of 4 subjects. One subject will receive placebo and three subjects will receive the lowest dose of the test compound (0.5 mg.kg⁻¹).

The second treatment block will also consist of 4 subjects. One subject will receive placebo and 3 subjects will receive 1.5 mg.kg⁻¹ of the test compound.

Session 3 will consist will consist of 10 subjects. 7 subjects will receive 5.0 mg.kg⁻¹ of the test compound and 3 subjects will receive placebo.

Session 4 will consist of 4 subjects. One subject will receive placebo and 3 subjects will receive 10.0 mg.kg⁻¹

Session 5 will consist of 9 subjects. If no effect is seen at 10.0 mg.kg⁻¹ in session 4 a further 4 subjects will receive 10.0 mg.kg⁻¹, 3 subjects will receive 15.0 mg.kg⁻¹ and two will receive placebo.

Session 6 will consist of 7 subjects. If no effect is seen at 15.0 mg.kg⁻¹ in Session 5 a further 4 subjects will receive 15.0 mg.kg⁻¹ and 3 subjects will receive placebo.

In the final session, 7 females will receive the NOEL dose identified in males and 3 females will receive placebo. If a NOEL in males is not identified at doses up to and including 15 mg.kg⁻¹, further cohorts of men may be studied and dosing in females delayed until a NOEL is identified.

4.1.2 Primary and Secondary Endpoints

In the absence of any pre-defined hypothesis, it is not possible to define primary and secondary endpoints for this study. All safety measures (i.e. adverse events, vital signs, and laboratory parameters) will be examined for any dose related trends.

4.1.3 Justification of Dose and Design

The oral route has been chosen as the route of administration as it is a major potential route of exposure to the compound. The doses have been chosen

after consideration of data from observations in earlier animal and human studies

4.1.4 Criteria for Stopping Study

The study will be discontinued if any subject exhibits more than 25% inhibition of red blood cell ChE or plasma ChE at two consecutive timepoints, after completing that dose level or if a mean inhibition in any cohort of more than 15% is noted at two consecutive timepoints.

4.1.5 Criteria for Dose Escalation

Progression to the next higher dose level will be permitted only after full review of all safety data indicates that it is safe to do so.

Dose escalation will not occur if any subject shows any signs or symptoms of organophosphate toxicity or has $\geq 25\%$ inhibition from baseline of red cell ChE or plasma ChE at two consecutive timepoints without associated symptoms or signs or a mean inhibition in any cohort of $\geq 15\%$ at two consecutive timepoints is noted.

4.2 Informed Consent

Before admission to the study each volunteer will be informed of the nature and the risks of the study and written informed consent will be obtained from the volunteers (Appendix A). Each volunteer's general practitioner will be asked if they have any objections to their patient's participation before the start of the study (Appendix B).

4.3 Selection Of Study Population

Subjects will be healthy male and female volunteers selected from the panel of volunteers recruited by ICR. A total of 48 will complete the study.

Subjects will be screened for inclusion in the study, according to the criteria for inclusion up to 21 days before dosing.

4.3.1 Pre-study screen

The screening examination will consist of:-

1. Medical history.
2. Complete physical examination and vital signs (pulse rate (supine and erect), respiratory rate, blood pressure and oral temperature).
3. 12-lead ECG recording.
4. Haematology, clinical chemistry, plasma and RBC cholinesterase and urinalysis (Appendix C).
5. Hepatitis B: Hbs-Ag.
HIV infection: HIV antibody.
Hepatitis C: Hep C Ab.
6. Urine screening for drugs, including drugs of abuse (including cannabis).
7. Pregnancy test (last cohort of females)

4.3.2 Inclusion Criteria

- (a) Males and females 18 to 50 years of age.
- (b) No clinically important abnormal physical findings at the screening examination.
- (c) No clinically relevant abnormalities in the results of laboratory screening evaluation including plasma and RBC cholinesterase (Appendix C).
- (d) Normal ECG.
- (e) Normal arterial pressure (BP) and heart rate (HR). These will be measured after resting supine for 3 minutes. Normal BP is taken to be 100 to 150 mm Hg systolic and 50 to 90 mm Hg diastolic. Normal HR is taken to be 50 to 90 bpm. Erect heart rate will be measured after standing for one minute. Normal erect HR is taken to be 50 to 100 bpm.
- (f) Body weight between 50 and 100 kg and within +/-15% of ideal body weight as given in the table of Appendix D.
- (g) Able to communicate well with the 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.

4.3.3 Exclusion Criteria

- (a) Administration of any investigational test compound in the period 0 to 3 months before entry to the study (0 to 4 months if the previous investigational test compound was a new chemical entity).
- (b) A need for any medication during the period 0 to 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 to 12 weeks before entry to 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 are not taking adequate contraceptive precautions.
- (j) Females with a positive pregnancy test.
- (k) Smokers who cannot abstain from smoking from 2 hours predose to 8 hours post dose.
- (l) Any subject with a resting pulse of <45 bpm, a systolic BP of <100 mm Hg or a PR interval on ECG of >210 ms.
- (m) Any subject who has had exposure to anti-cholinesterases (including home pest control products) within one month of dosing.
- (n) All agricultural workers or pest control applicators.

4.3.4 Restrictions

No alcohol, caffeine or concomitant medications (apart from paracetamol or other medications used to treat adverse events) will be allowed during study.

4.3.5 Withdrawal Criteria

ICR will make every reasonable effort to complete the study. If a subject wishes to leave the study at any time, he/she will be permitted to do so.

Every reasonable effort will be made by ICR to complete a final assessment. ICR will advise the sponsor of the withdrawal of the subject from the study.

A subject may 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

Any subject discontinuing the study compound prematurely because of reasons 1 or 4 will be considered to have completed the study. Where subjects are withdrawn because of reasons 2 or 3 they may be replaced, if insufficient data is obtained.

4.3.5.1 Data to be collected on withdrawal

If a subject withdraws for non-medical reasons at short notice, the following procedure will be adopted:

- the medical risks of withdrawing from the study within 12 hours of dosing will be explained to the subject.
- a physical examination will be performed.
- the 24h postdose safety assessments (vital signs, ECG, haematology, clinical chemistry, and urinalysis) will be performed.
- subject should sign a "Discharge Against Medical Advice" form, if necessary.

4.4 Investigational Products

4.4.1 Product Description

The investigational product will be provided by the Sponsor.

The matching placebo will be provided by ICR.

The active ingredient of the investigational product is malathion.

The investigational product will be provided as a clear liquid, in a glass bottle to be stored in the dark at ambient temperature (15-25°C).

The placebo will be provided as lactose.

The formulation will be administered by the oral route.

Doses will be prepared by direct weighing of the bulk malathion liquid into a size zero hard gelatin capsule.

The target weight of malathion in each capsule will be based on the dose level and the body weight at screening of the individual volunteer.

Weighing of malathion into capsule shells will be undertaken on a maintained and calibrated electronic balance with a readability capability of 0.01 mg. Capsules will be filled to an accuracy of $\pm 5\%$ of target dose weight for each volunteer.

The weights of malathion transferred to each capsule shell for each volunteer will be independently verified by the pharmacist responsible for undertaking the dose calculation.

Sponsor personnel or independent monitors acting for the sponsor may also verify the weights of malathion transferred to each capsule shell during the dispensing exercise, or alternatively blinded summaries of proposed weighing may be transmitted to the client for their approval prior to the dispensing exercise being undertaken. Sponsor personnel or independent monitors acting for the sponsor who have witnessed dose preparation will not be permitted to monitor Case Record Forms to protect the integrity of the study.

As a minimum the pharmacy will prepare 2 reference samples from each dispensing exercise representing duplicates of the doses prepared for the heaviest and lightest volunteers being dosed for each group.

4.4.2 Sponsor's Responsibilities

The sponsor will supply a quantity of malathion taken from a fully characterised batch of malathion in an appropriate container.

This will be supplied with a Certificate of Analysis and a statement of the expiry or re-test date and should be received at ICR at least 7 working days before the start of the study.

The sponsor will provide the investigational product packaged to prevent contamination or deterioration during transport and storage.

The sponsor will have determined acceptable storage temperatures, conditions and times to be observed for the investigational product and will inform ICR of these. If reconstitution is a requirement the sponsor will advise ICR of the procedures required.

4.4.3 ICR's Responsibilities

The Investigational product will be stored under the control of the ICR Pharmacist in a secure facility appropriate for the advised storage conditions.

Investigational products that require re-packaging or preparation for administration to humans will be handled by applying appropriate GMP principles and will be labelled by the ICR pharmacist according to current standard operating procedures.

4.4.4 Reconciliation

An accountability record of utilisation will be maintained for the Investigational product.

Unused Investigational product will be disposed of, or returned in accordance with written instructions from the sponsor.

4.4.5 Blinding

This is a double blind, placebo controlled study. The allocation to active or placebo will be random and based on the randomisation code generated by the statistics department of Inveresk Research.

It will be the responsibility of Inveresk Research to ensure that blinding is kept during the study.

A copy of the randomisation code will be held by the ICR pharmacist who will prepare the doses and he/she will not disclose the contents of the code to any member of the study team (except in circumstances detailed under "Emergency Procedures")

4.4.6 Concomitant Therapy

No concomitant therapy with the exception of paracetamol or other medications deemed necessary to treat adverse events will be allowed during the study. Atropine sulphate and a cholinesterase reactivator (pralidoxime; 2-PAM) will be available for use in the highly unlikely event that cholinergic symptoms or signs are observed.

4.5 Measurement of Safety

4.5.1 Safety Variables

4.5.1.1 Vital Signs

Supine systolic and diastolic arterial pressure will be determined by sphygmomanometry. Standing and supine pulse rates will be determined by palpation. Respiratory rate will be determined manually and oral temperature will be determined using a mercury thermometer.

Measurements will be made at screening, day-1, predose (0) and 2, 4, 8 and 24 hours after dosing.

4.5.1.2 Electrocardiography (ECG)

A 12 lead ECG will be obtained at screening, -30min predose and 2, 4, 8 and 24h postdose. If a subject shows an apparently abnormal ECG at any stage, repeat tracings will be made with the abnormality followed to resolution and additional lead recordings taken as deemed relevant.

4.5.1.3 Laboratory Data (Haematology/Clinical Chemistry)

Laboratory tests will be performed at screening, predose (0) and 24h postdose. Blood samples for clinical chemistry and haematology will be collected in pre-heparinised tubes (5.0 ml) and in EDTA-coated tubes (3.0 ml) respectively. Analyses are shown in Appendix C.

Laboratory tests showing abnormal values for any subject will be repeated as often as deemed necessary by the clinical investigator until the test values return to accepted limits or until an explanation other than compound effect is given.

Any abnormality fulfilling the common toxicity criteria (Appendix E) will be treated as an Adverse Event.

4.5.1.4 Urinalysis

Urinalysis (Appendix C) will be performed at screening and 24h postdose. Any abnormality fulfilling the common toxicity criteria (Appendix E) will be treated as an Adverse Event.

4.5.1.5 Additional Safety Variables

Single Channel Continuous ECG Monitoring

Single channel continuous ECG monitoring will be performed using a bedside monitor from -30min predose to 4h postdose.

Any subject demonstrating sustained bradycardia i.e. <50 bpm. or tachycardia i.e. >110 bpm for more than 30 seconds, will be reviewed by the clinical investigator.

4.5.1.5 Adverse Events

4.5.1.5.1 Definitions

Adverse Event (AE)

An adverse event is any unwanted event occurring during the course of a clinical trial.

Serious Adverse Events (SAE)

These are defined as adverse events which are fatal or considered life-threatening, which require hospitalisation or prolong hospitalisation, cause permanent disability, cancer, congenital anomaly or overdose, or are considered serious for any other reason.

Adverse Drug Reaction (ADR)

An adverse drug reaction is any adverse event suspected to be caused by the trial compound.

Adverse events include any symptom, physical sign, syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen during the course of a clinical trial, after starting treatment, whether considered treatment related or not.

"Treatment" includes all investigational agents (including placebo) administered during the course of the study. This is regardless of the suspected cause of the event.

Adverse events may be volunteered spontaneously by the subject, or be discovered as a result of general questioning by the investigator or by physical examination.

Questions will be phrased so that they do not "lead" the subject into giving information which is not valid. They must be recorded on the Adverse Events Form of the subject's Case Record Form.

As far as possible, each adverse event must also be described by its duration (start date, time and duration), its severity (Appendix E), an assessment of its cause (the underlying study indication, coexisting disease, concomitant medication, the study medication, or others), its relationship to the study medication (not related, unlikely, possibly, probably, definitely), whether it influenced the course of the study medication, or whether it required specific therapy.

4.5.1.5.2 Qualification of Adverse Events

Severity

All adverse events must be rated on a 3-point scale of increasing severity (mild, moderate or severe).

4.5.1.5.3 Relationship to Trial Compound

The relationship to the trial compound of all adverse events will be categorised according to the following table:

Relationship to Trial Treatment or Test Compound

1	NOT RELATED	This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
2	UNLIKELY (must have two)	In general, this category can be considered applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test compound. An adverse event may be considered unlikely to be related if or when: 1) It does not follow a reasonable temporal sequence from administration of the test compound. 2) It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It does not follow a known pattern of response to the test compound.
3	POSSIBLY (must have two)	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test compound administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when: 1) It follows a reasonable temporal sequence from administration of the compound. 2) It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It follows a known pattern of response to the test compound.
4	PROBABLY (must have three)	This category applies to those adverse events which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test compound. An adverse event may be considered probably related if or when: 1) It follows a reasonable temporal sequence from administration of the compound. 2) It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the compound, yet compound-relatedness clearly exists (e.g., bone marrow depression, fixed compound eruptions, tardive dyskinesia). 4) It follows a known pattern of response to the test compound.
5	DEFINITELY (must have all)	This category applies to those adverse events which the investigator feels are incontrovertibly related to test compound. An adverse event may be assigned an attribution of definitely related if or when: 1) It follows a reasonable temporal sequence from administration of the compound. 2) It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It disappears or decreases on cessation or reduction in dose and recurs with re-exposure to compound. (Note: this is not to be construed as requiring re-exposure of the subject, however, a category of definitely related can only be used when a recurrence is observed). 4) It follows a known pattern of response to the test compound.

4.5.1.5.4 Notification

ICR will notify the ethics committee of all adverse events.

Any serious adverse event will be notified to the sponsor within 24 hours by telephone or fax. This will be followed up by a full written report within three days.

The event must also be recorded on the standard Adverse Events Form, as described above.

These reports are to be made whether or not the investigator considers the serious adverse events to be related to the investigational compound.

A full written report will include photocopies of results, consultant report(s), and a summary of the outcome of the reaction plus the investigator's opinion of compound relationship to the serious adverse event(s).

4.5.1.5.5 Emergency Procedures

Emergency equipment and drugs (including atropine and 2-PAM) will be available for use by medical staff. In the unlikely event that they are required, their use will be documented.

Atropine antagonises the effects of accumulated acetylcholine and is the specific antidote for organophosphate toxicity. 2-PAM is a cholinesterase reactivator.

Additional supplies of atropine will be available at ICR for the duration of the study.

Atropine sulphate IV will be administered in increments of 0.6 mg up to a maximum of 3 mg by the clinical investigator in the event of severe symptoms/signs of organophosphate toxicity.

Atropine sulphate must be administered at the discretion of the clinical investigator in the following situations:-

- Symptomatic bradycardia (<50 bpm)
- Bradycardia (<50 bpm) associated with hypotension (systolic BP <90 mmHg).
- PR interval >240 ms or evidence of heart block.
- Worsening of muscle fasciculation or evidence of respiratory depression.

Atropine sulphate administration should be considered if the following occur:-

- Moderate/severe nausea/vomiting/diarrhoea/abdominal cramps causing distress.
- Moderate/severe muscle or tongue fasciculation causing distress.
- Severe sweating or salivation causing distress.
- Any combination of the above.

Copies of the randomisation schedule will be held at Inveresk Research Regulatory Affairs Department in the project file and at the investigational site in sealed envelopes in study file 3. The Study Director may request that the envelope be opened in the event of an emergency.

4.5.1.5.6 Follow up of subjects experiencing adverse events.

Subjects who experience adverse events will be followed up until resolution or until a non compound related cause has been established.

4.6 Measurement of Effects

Cholinesterase assay of Blood Samples

Samples will be collected during the course of the study at the following times:

Screening, Days -9, -7, -5, -2, -1 and -30min predose. All samples before dosing will be taken in the morning, at the same time of day if possible.

Blood (4.5 ml) will be collected into EDTA tubes from each subject and the tubes will be placed in ice.

After centrifugation the plasma and RBC fractions will be separated into 2 aliquots each. Samples up to 4h post dose will be transported to the lab at ERC on wet ice and assayed the same day. Samples later than 4h post dose will be centrifuged, separated and stored frozen overnight for assay on the next day.

The collected plasma and RBC samples will be assayed for cholinesterase (ChE) activity according to IR/SOP/CLC/636 and IR/SOP/CLC/920.

4.7 Collection of Samples for Analysis of Malathion Concentrations

4.7.1 Blood Samples

Samples will be collected for measurement of malathion and its malaoxon metabolite concentrations at the following timepoints:

Pre-dose (0), 1, 2, 4, 8, 12, 24, 48 and 72h post-dose

Blood (ca 14 ml) will be collected via a cannula or by repeated venepuncture into two 7 ml lithium heparin tubes. Plasma will be obtained by centrifugation. A minimum of 5 ml of plasma is required for analysis, therefore the plasma obtained from both blood tubes will be pooled as 1 plasma sample and stored at -70°C. Samples will be transferred frozen in dry ice to:

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Department of Bioanalytical Chemistry
Inveresk Research
Tranent
EH33 2NE
SCOTLAND

Analytical methods and other details of the analysis will be included in separate protocols.

4.7.2 Urine Samples

Urine will be collected for measurement of malathion mono - and di-carboxylic acids and dimethyl phosphate, dimethyl thiophosphate and dimethyl dithiophosphate at the following times:

-12-0h (pre-dose), 0-12, 12-24 and 24-48h post-dose.

Urine volume will be measured and 2 x 20ml aliquot from each period will be frozen at -70°C until analysis. Samples will be transferred frozen in dry ice to:

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Analytical methods and other details of the analysis will be included in a separate protocol.

4.8 Practicalities

**4.8.1 Schedule of Events
(see overleaf)**

SCHEDULE OF EVENTS

	Pre-study	Pre-dose		Times after dosing (h)										Post Study Days			
		Day -1	-30min	0	1	2	4	8	12	24	48	48 (i.e. +72h)	4	7	14		
Informed Consent		X															
History	X																
Physical Examination	X																
Compound Admin				X							X						
Haematology	X			X													
Clinical Chemistry	X			X													
Urinalysis	X																
ECG (12 lead)	X		X					X									
Vital Signs	X	X		X		X	X	X									
Urine drug screen	X																
Virology	X																
Pregnancy Test (& cohort)	X	X															
pk blood sample				X	X	X	X	X	X	X	X	X	X	X	X		
Cholinesterase Assay					X	X	X	X	X	X	X	X	X	X	X		
Adverse Events																	
Test Compound Accountability																	
Continuous Single Channel ECG																	
Urine																	

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* samples for cholinesterase assay will also be collected on Days -9, -7, -5 and Day -2.

4.8.2 Prestudy

Subjects will be admitted to the clinic on the morning before the study. The continued good health of each subject will be confirmed by enquiry and a brief examination (vital signs) on admission to the clinical unit. Blood samples will be obtained for plasma and red cell cholinesterase. A urine pregnancy test will be undertaken on female subjects.

A medication history update will also be obtained. Subjects will take no food or drink from 23.00h.

4.8.3 Study Day

Subjects must refrain from smoking from 2 hours predose.

At approximately 08.00h, subjects will be given a standard breakfast.

Approximately five minutes following completion of breakfast the formulation test compound will be administered to subjects in the sitting position with 150 ml of water. The time of dosing will be recorded for each subject.

The subjects will remain seated or recumbent in the dosing area until approximately 8h postdose at which time they may be ambulant. Subjects in the habit of smoking may do so, after this timepoint.

Subjects will remain fasted until approximately 4h postdose when a light lunch will be taken. Water, fruit juice (not grapefruit juice) or decaffeinated drinks will be allowed on request from approximately 3h postdose.

Normal activities, but excluding strenuous exercise, will be permitted from 8h postdose.

Actual times of the main events will be recorded.

Subjects will not be permitted to take drugs on the study day or until after the last blood sample has been withdrawn on Day 14 and will be advised not to drink alcohol until after the visit at 72h post dose (i.e. Day 4).

4.8.4 Post-study

Before discharge (approximately 48h post dose) all subjects will have a physical examination. Any abnormalities in investigations will be followed to normality.

All subjects will attend for a post-study follow-up visit on the mornings of Days 4 (72h post dose), 7 and 14 to ensure continued well-being and completion of any outstanding enquiries/adverse events and for collection of a blood sample for measurement of malathion and metabolite (day 4 only) and plasma and RBC cholinesterase activity (days 4, 7 and 14).

4.8.5 Critical Phases

All critical phases of the study will be supervised by medical and nursing personnel. Any deviations from the protocol will be recorded. A physician should be present for at least 3h after dosing.

4.8.6 Conditions for Modifying or Terminating the Study

Protocol Amendments

All changes or revisions of this protocol will be documented, signed and dated by the Study Director. The reason for the amendment will be stated.

All amendments will be sent to the sponsor and the ethics committee for approval and will be retained to the original protocol.

4.8.7 Conduct of the Study

The study will be conducted in accordance with the guidelines set out in the Declaration of Helsinki, 1964, as amended by the 29th Medical World Assembly in Tokyo, 1975, the 35th Medical World Assembly in Venice, 1983, the 41st Medical World Assembly in Hong Kong, 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996.

The study will be undertaken on the understanding that it may be modified or abandoned at the sole discretion of the ICR clinical investigator after consultation with the sponsor.

4.8.8 Confidentiality

It is agreed that the information contained in this protocol and the results of the study will not be disclosed to others, without written authorisation from the sponsor, except to staff involved in the study.

5. STATISTICS AND DATA MANAGEMENT

5.1 Randomisation

A subject number will be assigned for all subjects who qualify for the study in accordance with exclusion and inclusion criteria. The number will be assigned using the lowest number available.

Subject numbers will be allocated according to the code 001-099 and replacement subjects will be identified by 901-999. Based upon a computer-generated randomisation, subjects will be assigned to one of the dose levels.

The randomisation code will be held by the Regulatory Affairs Department at Inveresk Research and the Pharmacy Department at ICR where it is required for dispensing purposes.

The blindness of the study will be broken on completion of the clinical phase and after all adverse events have been assigned (i.e. relationship to test compound determined) and coded.

Any request for issue of the code must be made in writing by the Study Director using a Randomisation Distribution Form. Two sets of sealed envelopes will be provided containing individual subjects codes and retained with the study filing at ICR. They may be used in case of an emergency by the supervising physician or after discussion with the sponsor. Any code break will be recorded and a copy kept with the study file.

The randomisation and disclosure envelopes will be produced by the Statistics and Data Management Department of Inveresk Research according to standard procedures.

5.2 Sample Size Justification

The sample size of 48 subjects (4 or 10 subjects per dose level) is considered appropriate for a study of this type. No formal sample size calculation was performed.

5.3 Data Recording

The Case Record Forms will be prepared by ICR and will be reviewed by the Statistics and Data Management Department of Inveresk Research.

All data obtained during the course of the clinical phase of the study will be recorded directly and legibly into the Case Record Form in black ink.

5.4 Data Management

Data management will be performed by the Statistics and Data Management Department at Inveresk Research.

Adverse events and medications will be coded using the WHO Adverse Reaction Terminology (1989 or a more recent version) and WHO Drug Reference List (1991 or a more recent version) respectively. Primary and secondary coding will be performed and discrepancies adjudicated by an independent third party.

All study data recorded in the Case Record Form (CRF), except clinical chemistry, haematology, RBC and plasma cholinesterase will be subjected to double data entry using a validated database programmed in a clinical data management system. On completion of data entry the data will be exported to SAS for further consistency and validation checks. Following comparison of the data entries, the database will be locked and the audit trail switched on ie a computerised log of all subsequent changes to the data will

be recorded. All data queries will be raised using Data Resolution Forms (DRFs) and will be resolved with the assistance of ICR medical staff.

On resolution of all data queries, the database will be closed and all study data will be exported to SAS (v6.07) for the production of data listings and summary tables.

Clinical chemistry, haematology, RBC and plasma cholinesterase data will be collected by the online clinical pathology system and electronically transferred to SAS for the production of summary tables and data listings.

Plasma test compound and metabolite concentration data will be doubly entered into ASCII files. The resulting datasets will be compared and the final dataset will be reformatted in SAS and subjected to a 100% check against the source data. Furthermore plasma test compound concentration data will be reformatted via SAS to an ASCII file suitable for importing into WinNonlin (v1.1 or a more recent version) (a pharmacokinetics modelling package). The reformatted data will be entered in hours and all pharmacokinetic parameters presented in terms of hours. Pharmacokinetic parameter values will be received in the form of WinNonlin ASCII file output. This file will be read into SAS.

Urine test compound and metabolite concentration data will be doubly entered into ASCII files. The resulting datasets will be compared and the final dataset will be reformatted in SAS and subjected to a 100% check against the source data.

All data listings for inclusion into the study report, except adverse events, will be subjected to 10% quality control checks against the CRFs. All adverse events listings and summary tables will be subjected to 100% quality control checks.

On issue of the final report, Inveresk Research standard SAS datasets, used for the purposes of reporting and analysis, will be transferred to the Sponsor, if required.

5.5 Statistical Methods

The statistical package SAS (v6.07 or a more recent version) will be used to produce all summary tables and data listings. The summary tables and data listings will be produced by the Statistics and Data Management Department at Inveresk Research.

Throughout this section it is assumed that data from the male placebo subjects from the different dosing cohorts will be combined.

In general terms, categorical data will be presented using counts and percentages, whilst continuous variables will be presented using the mean, standard deviation, median, minimum, maximum and number of subjects. In general, minima and maxima will be quoted to the number of decimal places as recorded in the CRF; means and standard deviations will be quoted to one further decimal place. Percentages will be rounded to one decimal place.

5.5.1 Demographics and Other Baseline Characteristics

The following demographic variables will be summarised by gender and dose level: race, age, height, weight, and physical examination (normal/abnormal by body system). No significance testing of demographic data will be performed.

5.5.2 Safety Parameters

The objective of the statistical analysis is to investigate the data for any effects of test material on clinical tolerability and laboratory safety parameters. All such parameters will be summarised by gender, dose level and timepoint. With the exception of RBC and plasma cholinesterase, no formal statistical analysis will be performed.

RBC and plasma cholinesterase will be summarised (ie mean, standard deviation, minimum, maximum and n) at each timepoint, including changes from baseline, by gender and dose level. Additionally, the percentage change from baseline at each timepoint will be tabulated by gender and dose

level and illustrated graphically. Baseline will be defined as the mean of all available predose values (ie days -9, -7, -5, -2, -1 and -30 minutes).

For the male data, percentage change from baseline for RBC cholinesterase and plasma cholinesterase will be analysed using a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (ie 1, 2, 4, 8, 12, 24, 48, 72h (day 4), day 7 and day 14) and dose level by timepoint interaction. Subject will be included as a random effect. At each timepoint separately, a test for linear trend with dose will be performed using a linear contrast. In addition, using the error variance from the ANOVA pairwise comparisons between placebo and each dose level will be carried out, at each timepoint, using Student's 't'-tests. At each timepoint, if the test for linear trend is significant at the 5% level then the pairwise comparisons at that timepoint will not be adjusted for multiple comparisons. If the test for linear trend is not significant at the 5% level, a Bonferroni adjustment will be applied to the pairwise comparisons at that timepoint (ie each comparison will be tested at the 1.7% significance level). Treatment group LSMeans (ie means adjusted for any imbalance in the model) will be presented together with the significance level of the 't'-tests and the test for linear trend. In addition, where a Bonferroni adjustment was applied, the significance level of the pairwise comparisons after adjustment will also be presented.

For the female data, percentage change from baseline for RBC cholinesterase and plasma cholinesterase will be using a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (ie 1, 2, 4, 8, 12, 24, 48, 72h (day 4), day 7 and day 14) and dose level by timepoint interaction. Subject will be included as a random effect. Using the error variance from the ANOVA, a comparison between placebo and active group will be carried out, at each timepoint, using a Student's 't'-test. Treatment group LSMeans (ie means adjusted for any imbalance in the model) will be presented together with the significance level of the 't'-test.

Distributional assumptions underlying the statistical analyses will be assessed as follows: Normality will be examined using a Shapiro-Wilk test while homogeneity of variance will be assessed by plotting the residuals against the predicted values for the model. If there is significant non-normality which cannot be resolved by transforming the data, the data will be analysed

excluding outliers. However, if the omission of outliers has no effect on the conclusions, the results of the full dataset only will be reported.

Vital signs will be summarised (ie mean, standard deviation, minimum, maximum and n) at each timepoint, including changes from baseline (ie predose), by gender and dose level. Additionally, the number of subjects with 'substantial' increases or decreases in blood pressure (> 20 mmHg) and heart rate (> 15 bpm) will be tabulated.

Laboratory parameters will be summarised at each timepoint including changes from baseline (ie predose), by gender and dose level. In addition, abnormal values outside normal ranges will be flagged in the data listings.

Descriptive statistical methods will be used to summarise the following data types by dose level and, where appropriate, timepoint:

Demographic details

ECG

Urinalysis

Adverse events

Plasma concentrations of metabolite

Urine test compound and metabolite concentrations

Throughout the study, all adverse events either observed by medical staff or professional collaborators, or reported by the subject spontaneously or in response to a direct question, will be evaluated by the investigator and noted in the adverse event section of the CRF.

Adverse events will be coded using the WHO Adverse Reaction Terminology. Adverse events will be reported by primary body system and preferred term. In the tabulations, counting will be performed by subject and not event ie a subject reporting the same event more than once will have that event counted only once. All adverse events commencing prior to dosing with study medication will be excluded from the summary tables but will be fully listed.

A data listing of all information relating to adverse events will be provided. This data listing will include all events a subject experiences. The following tables will also be provided, by gender and dose level:

- number and percentage of subjects with at least one adverse event;
- number and percentage of subjects with serious adverse events;
- number and percentage of subjects with test compound-related adverse events.

The following breakdown of adverse events will be provided, by gender and dose level:

- by body system, preferred term and severity;
- by body system, preferred term and relationship to test compound.

5.6 Pharmacokinetic Methods

A measured test compound concentration vs. time curve will be produced, in graphic and tabular form, for each subject on both linear/linear and log/linear scales. Mean test compound concentration vs time curves will also be presented for each dose level separately. Summary statistics (i.e mean, standard deviation, minimum, maximum, n and coefficient of variation) will be calculated for plasma concentrations for each time point and each dose level.

Pharmacokinetic parameter values will be estimated using WinNonlin pharmacokinetic software (v1.1 or a more recent version). Unless otherwise agreed with the sponsor, a non-compartmental model will be used to generate parameter estimates. The following pharmacokinetic parameter estimates will be calculated:

C_{max}(obs) the observed maximum concentration of test compound in plasma measured in a subject after dosing, determined by direct inspection of the plasma test compound concentration vs. time data.

T_{max}(obs) the time at which C_{max}(obs) was apparent, determined by direct inspection of the plasma test compound concentration vs. time data.

Tlag(obs) Lag time; the time delay between dosing and onset of absorption for test compounds dosed via extravascular routes, if apparent.

AUC (0-t) the area under the plasma test compound concentration vs. time curve from time zero to 't' hours (where 't' = the time point for the last sample on the pharmacokinetic profile in which test compound was detected) calculated using the linear or log/linear trapezoidal method.

AUC(0-∞) the area under the plasma test compound concentration vs. time curve from time zero to infinity: $[AUC(0-∞) = AUC(0-t) + (C_t/K_{el})]$, where C_t = the concentration of test compound for the last sample on the pharmacokinetic profile in which test compound was detected, and K_{el} = the terminal elimination rate constant, determined from the slope of the terminal elimination phase].

CL/F clearance: the apparent volume of the central compartment cleared of test compound per unit time after i.v. dosing (or per unit time and per unit of body weight if dosing data is entered as, for example, $mg.kg^{-1}$). The estimate does not account for the bioavailability (F, as a fraction of 1) and is therefore nominally divided by this value when test compound is given via extravascular routes.

T½el terminal elimination phase half-life

Summary statistics (ie mean, standard deviation, median, minimum, maximum, n) and will be presented for all pharmacokinetic parameters by dose level. In addition, geometric mean and coefficient of variation (based on the logarithmically transformed data) will be presented for AUC and $C_{max}(obs)$ by dose level. The coefficient of variation will be calculated using the following formula:

$$CV(\%) = [\exp(sd^2) - 1]^{1/2} \times 100$$

where sd = standard deviation of the logarithmically transformed data.

AUC(0-∞) values will be analysed for dose proportionality using analysis of variance techniques (ref. Gough et al). The following model will be fitted:

$$\log(\text{AUC}) = \mu + \beta \cdot \log(\text{Dose})$$

This is usually referred to as a power model because after exponentiation:

$$\text{AUC} = \alpha \cdot \text{Dose}^\beta$$

The estimate obtained for β is a measure of dose proportionality. Dose proportionality requires that $\beta=1$ (if $\beta = 0$ this implies dose-independent parameters). The estimate of β together with its 95% confidence interval (β_L, β_U) will be presented to quantify the degree of non-proportionality.

The increase in AUC(0-∞) for a two-fold increase in dose will be calculated as 2^β . The confidence interval for this ratio will be obtained by substituting β_L and β_U in the equation.

The assumption of a linear relationship between the log AUC(0-∞) and log dose will be tested using analysis of variance by partitioning the sums of squares for treatments into those for linearity and departures from linearity. If the departures from linearity are significant then the test for dose proportionality will not be performed.

The individual AUC values will be presented graphically by dose level, in addition the linear regression line fitted will be displayed. The mean AUC values along with the standard deviation from the mean will also be displayed graphically by dose level.

Reference: Gough K., Hutchison M., Keene O., Byrom B., Ellis S., Lacey L., and McKellar J. Assessment of Dose Proportionality: Report from the Statisticians in the Pharmaceutical Industry/Pharmacokinetics UK Joint Working Party. Drug Information Journal, Vol 29, pp1039-1048, 1995.

6. DOCUMENTATION

6.1 Reporting

6.1.1 Interim Reports

Interim reports detailing adverse events and other relevant safety data, including cholinesterase results will be provided after each treatment block. These reports will be unaudited.

6.1.2 Study Reports

ICR will prepare the final report which will contain details of all safety data. The format of the report will be that of the Sponsor/ICR. The sponsor will provide a copy of his SOP for use by ICR within six weeks.

After the completion of the experimental work a draft report will be despatched to the sponsor.

Upon receipt of approval or amendments, or 16 weeks from the date of issue of the draft report (if no amendments have been requested), three copies of the final report will be despatched.

6.1.3 Case Record Forms (CRF's)

CRFs will be prepared in two part NCR paper

Upon completion of the experimental work the top copy will be sent to the sponsor.

6.2 Archives

All data produced will be stored in the archives of Inveresk Research. All data will be stored for fifteen years but after 5 years the sponsor will be consulted regarding the continued storage of raw data which will be at additional cost to the sponsor

Samples that are unstable may be disposed of before fifteen years after consultation with the sponsor.

6.3 Data Protection

When personal data on subjects are stored or processed by computer, the data must be protected to prevent their disclosure to unauthorised third parties. The pertinent sections of the UK data protection laws will be complied with in full.

7. ETHICS REVIEW PROCEDURE

The protocol and details of the study will be submitted to the ethics review committee of Inveresk Research in advance of the study. The participation by the sponsor in the preparation of material for submission is required by ICR. The study will not proceed until the approval of the ethics review committee has been received.

The written approval of the committee will be retained as part of the study file.

All amendments will be submitted to the Ethics Committee Chairman for approval.

8. GOOD CLINICAL RESEARCH PRACTICE

Studies will be conducted in accordance with the Guideline for Good Clinical Practice (CPMP/ICH/135/95).

9. QUALITY ASSURANCE

Quality assurance inspections will be carried out during the conduct of the study by the Inveresk QA unit. Phases selected for inspection will include (but will not be limited to):

Pharmacy data review
Dose preparation
Dosing

On study observations
Review of on-study case record forms and associated data
Data entry, where appropriate
Statistical analysis
Clinical Pathology
Bioanalytical Chemistry
Both the draft report and the final report will be audited

Selection of subjects for audit will be at the discretion of the QA unit and may be varied at any time during the course of the audit. QA also reserve the right to conduct for cause inspection of any aspect of the study without prior notification.

Records of these inspections and audits will be documented and distributed to study management for review. Copies of internal Inveresk and any 3rd party QA consultant audit and inspection reports, with responses, will be provided by the sponsor Representative and QA unit upon completion of each audit.

The conduct of these inspections and audits will be carried out according to standard operating procedures by quality assurance personnel of Inveresk Research, who are independent of those responsible for the trial.

10. **SPECIAL CONDITIONS**

The study will proceed only after documented acceptance of the protocol by the sponsor has been received by ICR.

Except in the proven case of clinical malpractice, the sponsor will indemnify ICR against any claim made by or on behalf of volunteers or their dependants which may result from administration of the clinical trial material.

11. **TIMEPLAN**

Study start:	October 1998.
Clinical phase completed:	February 1999.
Issue of draft report:	TBC
Issue of final report:	TBC

APPENDIX A

Volunteer Consent Form

This agreement is between the volunteer _____ and Inveresk Clinical Research Limited (hereinafter referred to as ICR), and provides for the volunteer to take part in experiments, trial and/or tests of a chemical compound or compounds.
FOR ALL STUDIES

- 1) I, the undersigned voluntarily agree to take part in

Protocol No: 013177

Descriptive Study Title: A randomised double blind ascending single oral dose study with malathion to determine the no effect level on plasma and RBC cholinesterase activity.

I understand that the investigation will involve the administration of

Name(s) of compounds: Malathion

being the compound under test.

- 2) I have been given a full explanation by Dr _____ of the nature, purpose and likely duration of the study and what I will be expected to do and I have been advised about any discomfort and possible ill-effects on my health or well-being which he/she believes may result. The information document given to me is attached (Appendix A pages 49 of 69 to 52 of 69).
- 3) I have been given the opportunity to question Dr _____ on aspects of the study and have understood the advice and information given as a result.
- 4) I agree to Dr Freestone contacting my general practitioner (and teaching or university authority if appropriate) to make known my participation in the study and I authorise my general practitioner to report details of my relevant medical or drug history, in confidence.

APPENDIX A (Continued)

- 5) I agree to comply with any instruction given during the study and to cooperate faithfully with Dr Freestone and to tell him immediately if I suffer from any deterioration of any kind in my health or well-being or any unexpected or unusual symptoms however they may have arisen.
- 6) I agree that I will not seek to restrict the use to which the results of the study may be put and, in particular, I accept that they may be disclosed to regulatory authorities for compounds in the UK and elsewhere.
- 7) I understand that I am free to withdraw from the study at any time without needing to justify my decision.
- 8) The supervising doctor confirms that subject to overriding requirement of law necessitating the disclosure of documents relating to the study, the volunteer will not be referred to by name in any document concerning the study disclosed to any person not under the direct control of the supervising doctor;
- 9) ICR confirms that:
 - (i) I shall receive in consideration for completing the study, the sum of £450 from the supervising doctor and that I shall receive the sum in full if it is necessary for me to withdraw from the study for medical reasons associated with participation in it. If I withdraw from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if I withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising doctor;

APPENDIX A (Continued)

- (ii) In the event of my suffering any bodily injury caused directly by my participation in the study, compensation will be paid to me by the company without having to prove that the injury arose through negligence or that the study compound was defective as set forth in the Association of the British Pharmaceutical Industry "Guidelines for medical experiments in non-patient human volunteers".
- (iii) The amount of such compensation shall be calculated by reference to the amount of damages commonly awarded for similar injuries by an English court if liability is admitted, providing that such compensation may be reduced to the extent that I, by reason of contributory fault through my actions or my failure to act, am partly responsible for the injury.
- (iv) Any dispute or agreement as to the application of clause 9 (ii) shall be referred to an arbitrator to be agreed between myself and the company, or in the absence of agreement, to be appointed by the President of the Royal College of Physicians of London with power in the arbitrator to consult a barrister of 10 years standing in respect of any issue of law including the amount of damages to be awarded as payment of compensation;
- (v) The agreement shall be construed in accordance with English law and subject to clause 9 (ii), (iii) and (iv) above the English courts shall have sole jurisdiction over any dispute which may arise out of it.

APPENDIX A (Continued)

Signed by the volunteer :

Dated :

Signed for on behalf
of the company by
its duly authorised
representative:

Dated :

I confirm that I
have explained the
nature, purpose and
possible hazards of
the above trial to :

Signed :

Dated :

I confirm that I have witnessed the above explanation:

Signed :
Witness Signature

Dated :

(NB-It may be appropriate for the supervising doctor to fulfil the obligations of the
duly authorised representative for the company.

Since signing the consent, the volunteer information sheet has been changed. I confirm
that I have read the revised information (dated / /) and still consent to participation in
the study.

Signed :

Dated :

APPENDIX A (Continued)

Volunteer Information

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND RBC CHOLINESTERASE ACTIVITY.

Introduction

You are invited to take part in a study involving a pesticide called malathion proposed for use in controlling non-beneficial worms and insects which eat the plant leaves or fruit in a variety of fruit and vegetable crops, sugarbeets, cotton and ornamental plants.

Very small amounts of residue may exist at harvest on treated crops. To ensure the safety of these residues for human consumption, many studies have been performed in animals and one human study has already been conducted. These studies showed no side effects other than reducing the amount of an enzyme protein (cholinesterase) which breaks down a chemical substance (acetylcholine) in the body responsible for the transmission of nervous impulses. Large increases of acetylcholine in the nervous system can cause increased salivation, sweating, reduced blood pressure, nausea, vomiting and stomach cramps.

Although the mechanism of action of cholinesterase inhibitors is well understood, species differences do exist. This study is being conducted to reduce the uncertainties of species differences in determining a level of human exposure that causes minimal reduction of blood cholinesterase levels. A significant reduction of cholinesterase in the nervous system is required before any clinical effects are observed. The results of this study will further confirm that the use of malathion does not pose an unreasonable risk to either workers or consumers.

Aim

The aim of the study is to determine the highest dose at which no significant reduction of blood cholinesterase occurs.

Dose

The doses to be given are 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹ body weight and placebo (inactive compound). A maximum of 48 of you will be tested in 7 groups. In the first group, one subject will receive placebo and three subjects the lowest dose of active compound (0.5 mg.kg⁻¹). In the second group, one subject will receive placebo and three subjects will receive 1.5 mg.kg⁻¹. In the third group three subjects will receive placebo and seven subjects will receive 5.0 mg.kg⁻¹. In the fourth group, one subject will receive placebo and three subjects will receive 10.0 mg.kg⁻¹. In the fifth group two subjects will receive placebo, four subjects will receive 10.0 mg.kg⁻¹ and three subjects will receive 15.0 mg.kg⁻¹. In the sixth group three subjects will receive placebo and four subjects will receive 15.0 mg.kg⁻¹. When the maximum dose that causes no significant inhibition of blood cholinesterase in men has been identified, this dose will be given to a group of 10 women (7 to receive active compound and 3 placebo).

Allocation to receive active compound or placebo (inactive compound) will be randomised. The compound or placebo will be administered as capsules by mouth.

APPENDIX A (Continued)

Volunteer Information

Side Effects

While it is not anticipated that at the low doses to be used, any effects other than a reduced level of blood cholinesterase will occur, information from clinical effects caused by similar compounds at much higher dose levels suggests the following effects are possible:

Initial symptoms are headache and nausea, followed by a feeling of chest tightness and coughing. At dose levels much higher than those being used in this study other possible symptoms include vomiting, diarrhoea, abdominal pain, blurred vision, weakness, sweating, constricted pupils, excess saliva production, slow pulse, and involuntary muscle twitching. It is highly unlikely that any of these effects will occur.

In some animal studies (but not all) it has been shown that lifetime exposure to 1600 mg/kg daily of malathion (ie more than 100 times the maximum dose in this study every day for a lifetime) in sensitive species caused an increased incidence of liver cancer. The dose level and duration of treatment bears no relationship to the single low doses to which volunteers will be exposed in this study.

Procedure

You will attend ICR for screening within 21 days of the start of the study. At screening, a complete medical history will be taken and you will have a complete physical examination, recordings of your pulse and blood pressure obtained, an ECG recorded (tracing of your heart's electrical activity) and blood samples taken for various safety tests. Samples of blood will also be taken to test for hepatitis B, hepatitis C and HIV, the virus that can cause AIDS. Urine will also be tested including a test for drugs of abuse.

Your GP will be informed of your participation and asked to confirm your medical history. If there are any objections expressed by your GP you will be excluded from the study.

Once you have successfully passed the screening examination, including acceptable blood and urine test results, your co-operation with the following will be required:

1. Up to a total of 48 of you will be studied.
2. If you smoke you must be able to abstain from smoking from 2h predose to 8h postdose.
3. You will require to attend the clinic for 4 outpatient visits on 9, 7, 5 and 2 days prior to dosing when a blood sample will be taken.
4. You will then be resident in the clinic on one occasion for 3 nights and will then return for 3 further outpatient visits 3, 6 and 13 days after dosing.
5. You will be admitted to the clinic between 10am and mid-day on the morning preceding the day of dosing. A brief examination including vital signs will be performed, and a blood sample taken for measurement of cholinesterase. A urine pregnancy test will be undertaken on female subjects. You will take no food or drink from 2300h.

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APPENDIX A (Continued)

Volunteer Information

6. On the day of dosing, you will be given breakfast and 5 min after completion you will receive either the active compound or placebo with 150 ml of water in the sitting position. You will then be required to remain seated or recumbent until 8h after dosing. You will be allowed water, fruit juice or decaffeinated drinks from approximately 3h after dosing. A light lunch will be provided approximately 4h after dosing. Normal activities excluding strenuous exercise will be allowed from approximately 4h after dosing.
7. Before dosing, a cannula (plastic tube inserted by a needle into a vein) will be inserted into your arm in order to allow samples of your blood to be obtained at regular intervals throughout the day of dosing. A needle and syringe can be used repeatedly to obtain blood samples if preferred. Repeated blood tests and cannulae can cause soreness and bruising of the arms or even, rarely, blockage of a vein, but those problems usually clear up within a few days to a few weeks.
8. Blood pressure, heart rate and a tracing of your heart's electrical activity will be recorded at intervals before and after dosing.
9. You will also be monitored for the presence or absence of the clinical signs listed in the introduction. A continuous recording of your heart's electrical activity will be displayed on a bedside monitor from 30mins before dosing to 4h after dosing.
10. You will be discharged 48h after dosing. Before discharge, a physical examination will be performed and blood samples will be taken for safety assessments.
11. You will return to the clinic in the morning 3, 6 and 13 days after dosing when a blood sample will be taken and to ensure continued well-being and for completion of any outstanding enquiry/adverse events.
12. Approximately 235 ml of blood will be taken during the study (compared with 480ml which is a standard blood donation).
13. You should avoid medication (including over-the-counter products) for 5 days before the start of the study.
14. You will not be allowed to take alcohol or other drugs on each resident study day or until after the last blood sample has been taken. It is recommended that you should refrain from alcohol as far as possible until the follow-up visit on Day 14.

If anything abnormal occurs, judged by the supervising clinician, or if laboratory investigations change, you may be withdrawn from the study. In addition, you may withdraw at any time without needing to justify your decision. (You are strongly advised not to leave the clinical unit within 24h after dosing as this may involve risk to your health). If you decide to withdraw before completion of the study a medical examination including blood pressure and heart rate, blood sampling, urine sampling and an ECG will be performed.

You should inform the supervising physician of any symptoms. After the study is over, you will be given a telephone number to call if you have any questions or worries.

APPENDIX A (Continued)

Volunteer Information

It is essential that you should adhere to all of these requirements. The supervising physician will be pleased to supply any further information at any time.

All information will be treated in a confidential manner but anonymised data will be seen by authorised persons involved in the study and possibly by compound regulating authorities.

Supervising Physicians

Dr J Dickson and Dr S Freestone
Inveresk Clinical Research
Riccarton
Edinburgh
EH14 4AP Tel: (0131) 451 5080

APPENDIX B

Letter to Volunteer's General Practitioner

**STEPHEN FREESTONE MD, FRCPEdIn
MEDICAL DIRECTOR**

Name and Address of Doctor

Date:

Dear Dr

Human Volunteer Study: A Randomised Double Blind Ascending Single Oral Dose Study with malathion to determine the No Effect Level on Plasma and RBC Cholinesterase activity.

Dates of Study:

Your Patient:

D.O.B.:

Inveresk Clinical Research Limited carries out compound evaluation and research as a preparation for regulatory agency submissions and product licence applications in the UK and other countries of the world. As part of its programme, volunteers help from time to time with the investigations.

We have invited your patient to take part in the above experimental study which will be carried out under full medical supervision at the Clinical Unit of Inveresk Clinical Research. He has consented to my contacting you. I enclose a copy of the signed volunteer consent form.

A brief statement of the nature of the test compound is contained in the attached summary.

If you have any medical objections to your patient taking part in this study, we would be grateful if you could inform us of your objections as soon as possible. In any event, it would be most helpful to us if you could complete and return the enclosed questionnaire.

Please address any account you may wish to send to Inveresk Clinical Research Limited. Fees should be in accordance with those recommended by BMA guidelines. Thank you in anticipation for your co-operation.

Yours sincerely

DR S FREESTONE

APPENDIX B (Continued)

**STEPHEN FREESTONE MD, FRCPedIn
MEDICAL DIRECTOR**

Date:

Study No: 013177

Patient's Name:

Date of Birth:

General Practitioner:

MEDICAL HISTORY

YES NO
(Tick as Appropriate)

Disorders of the central nervous system: ☐ ☐
Disorders of the cardiovascular system: ☐ ☐
Disorders of the respiratory system: ☐ ☐
Diseases and disorders of the alimentary system: ☐ ☐
Disorders of the genito-urinary system: ☐ ☐
Psychiatric disorders: ☐ ☐
Alcohol/drug abuse: ☐ ☐
Other (e.g. diabetes mellitus, thyroid disorder, allergic): ☐ ☐
Recent prescribed drug treatment: ☐ ☐
Any evidence of adverse drug reaction: ☐ ☐
Do you have any objections to your patient participating in this trial? ☐ ☐

If Yes, please specify

Has your patient participated in a trial of an unlicensed drug in the last 6 months?

Yes ☐ No ☐ Don't Know ☐

How long do your records cover the medical history of this patient? ____ Years

Signed: _____ Date: _____
General Practitioner

Authorisation Stamp: _____

Please note: This statement does not imply your consent. Responsibility for a subject entering a study is between the volunteer and ICR.

FOR OFFICIAL USE ONLY

Comments:	Signature:	Date of Review:

APPENDIX B (Continued)

STEPHEN FREESTONE MD, FRCPEdIn
MEDICAL DIRECTOR

Date:
Patient's Name:
Date of Birth:
General Practitioner:

Study No: 013177

MEDICAL HISTORY

A full questionnaire has been completed on the above patient within the last twelve months.

Has there been a significant change in this patient's medical history since _____?

Yes ☐ No ☐ (Tick as appropriate)

If yes, please comment: _____

Do you have any objections to your patient participating in this trial?

Yes ☐ No ☐ (Tick as appropriate)

If Yes, please specify: _____

Has your patient participated in a trial of an unlicensed drug in the last 6 months?

Yes ☐ No ☐ Don't Know ☐

How long do your records cover the medical history of this patient? _____ years

Signed: _____ Date: _____

General Practitioner

Authorisation Stamp: _____

Please note: This statement does not imply your consent. Responsibility for a subject entering a study is between the volunteer and ICR.

FOR OFFICIAL USE ONLY

Comments:	Signature:	Date of Review:
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AGE RANGE 18-50**APPENDIX C****Methods, Abbreviations and Units in Laboratory Investigations****Haematology**

<u>Parameter</u>	<u>Method</u>	<u>Normal Range</u>	<u>Units</u>
Haemoglobin: (Hb)	Technicon H1 Analyser Bayer UK Ltd	12.8 - 16.3 % 10.8 - 15.3 &	g/dl
Total Red Blood Cell Count: (RBC)	Technicon H1 Analyser Bayer UK Ltd	4.17 - 5.55 % 3.60 - 5.06 &	x10 ¹² /l
Haematocrit: (Hct)	Technicon H1 Analyser Bayer UK Ltd	0.382 - 0.487 % 0.321 - 0.457 &	l/l
Mean Cell Haemoglobin: (MCH)	Technicon H1 Analyser Bayer UK Ltd	27.4 - 32.3 % 27.5 - 32.2 &	pg
Mean Cell Volume: (MCV)	Technicon H1 Analyser Bayer UK Ltd	81.4 - 96.3 % 82.0 - 97.8 &	fl
Mean Cell Haemoglobin Concentration: (MCHC)	Technicon H1 Analyser Bayer UK Ltd	31.9 - 35.6 % 31.6 - 34.9 &	g/dl
White Blood Cell Count: (WBC)	Technicon H1 Analyser Bayer UK Ltd	3.62 - 10.49 % 3.84 - 10.82 &	x10 ⁹ /l

AGE RANGE 18-50**APPENDIX C****Methods, Abbreviations and Units in Laboratory Investigations****Haematology**

<u>Parameter</u>	<u>Method</u>	<u>Normal Range</u>	<u>Units</u>
Differential White Cell Count			
Neutrophils: (Neut)	Technicon H1 Analyser Bayer UK Ltd	1.58 - 7.47 % 1.86 - 7.43 %	x10 ⁹ /l
Lymphocytes: (Lymph)	Technicon H1 Analyser Bayer UK Ltd	1.01 - 3.03 % 1.09 - 3.16 %	x10 ⁹ /l
Monocytes: (Mono)	Technicon H1 Analyser Bayer UK Ltd	0.18 - 0.75 % 0.16 - 0.67 %	x10 ⁹ /l
Eosinophils: (Eos)	Technicon H1 Analyser Bayer UK Ltd	0.04 - 0.47 % 0.03 - 0.48 %	x10 ⁹ /l
Basophils: (Baso)	Technicon H1 Analyser Bayer UK Ltd	0.02 - 0.09 % 0.02 - 0.11 %	x10 ⁹ /l
Large Unclassified Cells (LUC)	Technicon H1 Analyser Bayer UK Ltd	0.07 - 0.32 % 0.09 - 0.36 %	x10 ⁹ /l
Platelets: (Plat)	Technicon H1 Analyser Bayer UK Ltd	126 - 331 % 108 - 362 %	x10 ⁹ /l

AGE RANGE 18-50APPENDIX CMethods, Abbreviations and Units in Laboratory InvestigationsClinical Chemistry

<u>Parameter</u>	<u>Method</u>	<u>Normal Range %/g</u>	<u>Units</u>
Urea	Sampson E J <i>et al</i> (1980) Clin Chem <u>26</u> 816 - 826	1.7 - 8.3	mmol/l
Glucose: (Glu)	Anon (1983) J Clin Chem Clin Biochem <u>21</u> 749 - 760	3.8 - 6.5	mmol/l
Aspartate Amino- Transferase: (AST)	Bergmeyer, H.U <i>et al</i> , (1986) J Clin Chem Clin Biochem <u>24</u> 497 - 510	5 - 43	iu/l
Alanine Amino- Transferase: (ALT)	Bergmeyer, H.U <i>et al</i> , (1986) J Clin Chem Clin Biochem <u>24</u> 497 - 510	8 - 45	iu/l
Lactate Dehydrogenase: (LDH)	Bergmeyer H U (1975) Z Clin Chem Biochem <u>13</u> 507	229 - 460	iu/l
Sodium: (Na)	Tietz N (1986) Text Book of Clinical Chemistry, WB Saunders Company	135- 150	mmol/l
Potassium: (K)	Tietz N (1986) Text Book of Clinical Chemistry, WB Saunders Company	3.3 - 4.8	mmol/l
Chloride: (Cl)	Tietz N (1986) Text Book of Clinical Chemistry, WB Saunders Company	97 - 109	mmol/l
Total Protein: (TP)	Friedman R <i>et al</i> (1980) Clin Chem <u>26</u> 4	66 - 87	g/l

AGE RANGE 18 - 50**APPENDIX C****Methods, Abbreviations and Units in Laboratory Investigations****Clinical Chemistry**

<u>Parameter</u>	<u>Method</u>	<u>Normal Range %/±</u>	<u>Units</u>
Albumin: (Alb)	Friedman R et al (1989) Effects of Disease on Clinical Laboratory Tests, AACCC Press, Washington DC	41 - 54	g/l
Creatinine: (Crea)	Butler A R (1975) Clin. Chem. Acta. 59 227-232	77-136	µmol.l ⁻¹
T.Bilirubin: (T.Bil)	Henry R J et al (1974) Clin Chem Principles and Techniques, Harper and Row 1059	3.7 - 21.0	umol/l
Gamma Glutamyl Transpeptidase: (GGT)	Young D S et al (1975) Clin Chem 21 5	3 - 60	Iu/l
Plasma Cholinesterase (Plasma ChE)	Ellman, G.L., et al, Biochem Pharmacol, 7:78, (1961)	3000-8000	Iu.l ⁻¹
Red Cell Cholinesterase (RBC ChE)	Ellman, G.L., et al, Biochem Pharmacol, 7:78, (1961)	4000-12500	Iu.l ⁻¹

APPENDIX C (Continued)

Urinalysis

Tests:	pH
	Specific Gravity
	Protein
	Glucose
	Ketones
	Bilirubin
	Blood
	Urobilinogen

All tests are performed using:	Multistix
	Ames Division
	Mill Laboratories Ltd
	Stoke Poges
	Slough SL2 2LY

Urine samples showing the presence of protein or blood will be spun and a sample of the spun deposit examined microscopically.

APPENDIX D

Metropolitan Life Insurance

Height and Weight Standards

Males

HEIGHT (cm)	WEIGHT IN INDOOR CLOTHING (kg)		
In shoes 2.5cm heels	SMALL FRAME	MEDIUM FRAME	LARGE FRAME
157.5	58.1-60.1	59.5-64.0	62.6-68.0
160.0	59.0-61.7	60.4-64.9	63.6-69.5
162.6	59.9-62.7	61.7-65.8	64.5-70.8
165.1	60.8-63.6	62.2-67.2	65.4-72.6
167.6	61.7-64.5	63.1-68.6	66.3-74.5
170.2	62.7-65.8	64.5-69.9	67.6-76.3
172.7	63.6-67.2	65.8-71.3	69.0-78.1
175.3	64.5-68.6	67.2-72.6	70.4-79.9
177.8	65.4-69.9	68.6-74.0	71.7-81.7
180.3	66.3-71.3	69.9-75.4	73.1-83.5
182.9	67.6-72.6	71.3-77.2	74.5-85.4
185.4	69.0-74.5	72.6-79.0	76.3-87.2
188.0	70.4-76.3	74.5-80.8	78.1-89.4
190.5	71.7-78.1	75.8-82.6	79.9-91.7
193.0	73.5-79.9	77.6-84.9	82.2-94.0

These are 1983 Metropolitan Life Insurance company weight tables by height and size of frame, for people aged 25 to 59, in shoes, wearing 2.3kg of indoor clothing for men, 1.4kg for women.

APPENDIX D (continued)

Metropolitan Life Insurance

Height and Weight Standards

Females

HEIGHT (CM) In shoes 5cm heels	WEIGHT IN INDOOR CLOTHING (Kg)		
	SMALL FRAME	MEDIUM FRAME	LARGE FRAME
147.3	46.3-50.4	49.5-54.9	53.6-59.5
149.9	46.7-51.3	50.4-61.3	54.5-60.8
152.4	47.2-52.2	51.3-57.2	55.4-62.2
154.9	48.1-53.6	52.2-58.6	56.8-63.6
157.5	49.0-54.9	53.6-59.9	58.1-64.9
160.0	50.4-56.3	54.9-61.3	59.5-66.7
162.6	51.6-57.7	56.3-62.7	60.8-68.6
165.1	53.1-59.0	57.7-64.0	62.2-70.4
167.6	54.5-60.4	59.0-65.4	63.6-72.2
170.2	55.8-61.7	60.4-66.7	64.9-74.0
172.7	57.3-63.1	61.7-68.0	66.3-75.8
175.3	58.6-64.5	63.1-69.5	67.6-77.2
177.8	59.9-65.8	64.5-70.8	69.0-78.5
180.3	61.3-67.2	65.8-72.2	70.4-79.9
182.9	62.7-68.6	67.2-73.5	71.7-81.3

APPENDIX E**TOXICITY RATING SCALE**

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
HAEMATOLOGY				
Haemoglobin	9.5-10.5 g.dl ⁻¹	8.0-9.4 g.dl ⁻¹	6.5-7.9 g.dl ⁻¹	<6.5 g.dl ⁻¹
Absolute Neutrophil Count	1.00-1.50 x 10 ⁹ l ⁻¹	0.70-0.99 x 10 ⁹ l ⁻¹	0.50-0.69 x 10 ⁹ l ⁻¹	<0.50 x 10 ⁹ l ⁻¹
Platelet Count	75-99 x 10 ⁹ l ⁻¹	50-74 x 10 ⁹ l ⁻¹	20-49 x 10 ⁹ l ⁻¹	<20 x 10 ⁹ l ⁻¹
CLINICAL CHEMISTRY				
Hyponatraemia	130-134 mmol.l ⁻¹	123-129 mmol.l ⁻¹	116-122 mmol.l ⁻¹	<116 mmol.l ⁻¹
Hypernatraemia	150-154 mmol.l ⁻¹	155-158 mmol.l ⁻¹	159-165 mmol.l ⁻¹	>165 mmol.l ⁻¹
Hypokalaemia	3.0-3.3 mmol.l ⁻¹	2.5-2.9 mmol.l ⁻¹	2.0-2.4 mmol.l ⁻¹	<2.0 mmol.l ⁻¹
Hyperkalaemia	5.6-6.0 mmol.l ⁻¹	6.1-6.5 mmol.l ⁻¹	6.6-7.0 mmol.l ⁻¹	>7.0 mmol.l ⁻¹
Hypoglycaemia	3.1-3.6 mmol.l ⁻¹	2.2-3.0 mmol.l ⁻¹	1.7-2.1 mmol.l ⁻¹	<1.7 mmol.l ⁻¹
Hyperglycaemia *	6.5-8.9 mmol.l ⁻¹	9.0-13.9 mmol.l ⁻¹	14.0-28.0 mmol.l ⁻¹	>28.0 mmol.l ⁻¹

* Hyperglycaemia: non-fasting and no prior diabetes.

TOXICITY RATING SCALE (cont.)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
Hyperbilirubinemia	25.0-31.5 mmol.l ⁻¹	31.6-52.5 mmol.l ⁻¹	52.6-105.0 mmol.l ⁻¹	>105.0mmol.l ⁻¹
Urea (18-40y) (40+)	10.4-20.8 mmol.l ⁻¹	20.9-41.5 mmol.l ⁻¹	41.6-83.0 mmol.l ⁻¹	>83.0 mmol.l ⁻¹
	11.5-23.0 mmol.l ⁻¹	23.1-46.0 mmol.l ⁻¹	46.1-92.0 mmol.l ⁻¹	>92.0 mmol.l ⁻¹
Creatinine (18-40y) (40+)	134-183 µmol.l ⁻¹	184-366 µmol.l ⁻¹	367-732 µmol.l ⁻¹	>732 µmol.l ⁻¹
	150-204 µmol.l ⁻¹	205-408 µmol.l ⁻¹	409-816 µmol.l ⁻¹	>816 µmol.l ⁻¹
AST	54-107 iu.l ⁻¹	108-215 iu.l ⁻¹	216-430 iu.l ⁻¹	>430 iu.l ⁻¹
ALT	56-112 iu.l ⁻¹	113-225 iu.l ⁻¹	226-450 iu.l ⁻¹	>450 iu.l ⁻¹
GGT	75-150 iu.l ⁻¹	151-300 iu.l ⁻¹	301-600 iu.l ⁻¹	>600 iu.l ⁻¹
Alkaline Phosphatase	349-697 iu.l ⁻¹	698-1395 iu.l ⁻¹	1396-2790 iu.l ⁻¹	>2790 iu.l ⁻¹
URINALYSIS				
Proteinuria	1+ or <0.3% or <3 g.l ⁻¹ or 200 mg - 1 gm loss.day ⁻¹	2-3+ or 0.3-1.0% or 3-10 g.l ⁻¹ or 1-2 gm loss.day ⁻¹	4+ or >1.0% or >10 g.l ⁻¹ or >2-3.5 gm loss.day ⁻¹	Nephrotic syndrome or >3.5 gm loss.day ⁻¹
Gross Haematuria	microscopic only	gross, no clots	gross + clots	obstructive or requires transfusion

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
RESPIRATORY				
Cough - for aerosol studies	transient - no Rx	treatment associated cough; local Rx	uncontrolled	
Bronchospasm acute	transient: no Rx, FEV1 <80%-70% (or peak flow)	req. Rx; normalises with bronchodilator; FEV1 50%-70% (or peak flow)	no normalisation w/bronchodilator; FEV1 25%-50% (or peak flow), retractions	cyanosis; FEV1 <25% (or peak flow) or intubated
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring therapy
NEURO/NEUROMUSCULAR				
Neuro-cerebellar	slight incoordination OR dysdiadochokinesia	intention tremor OR dysmetria OR slurred speech; nystagmus	locomotor ataxia	incapacitated
Neuro-psych/mood			severe mood changes requiring medical intervention	Acute psychosis requiring hospitalisation
Paraesthesia (burning, tingling etc.)	mild discomfort; no Rx required	mod discomfort; non-narcotic analgesia required	severe discomfort; OR narcotic analgesia required with symptomatic improvement	incapacitating; OR not responsive to narcotic analgesia

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
Neuro-motor	mild weakness in muscle of feet but able to walk and/or mild increase or decrease in reflexes	mod weakness in feet (unable to walk on heels and/or toes), mild weakness in hands, still able to do most hand tasks and/or loss of previously present reflex or development of hyperreflexia and/or unable to do deep knee bends due to weakness	marked distal weakness (unable to dorsiflex toes or foot drop) and mod proximal weakness eg in hands interfering with ADLs and/or requiring assistance to walk and/or unable to rise from chair unassisted	Confined to bed or wheel chair because of muscle weakness
Neuro-sensory	mild impairment (dec sensation eg vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution	mod impairment (mod dec sensation eg vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	Severe impairment (dec or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (ie upper and lower extremities)	Sensory loss involves limbs and trunk
Mood	mild anxiety or depression	therapy required for mod. depression OR mod. anxiety	needs assistance due to severe depression OR mania OR anxiety	acute psychosis or incapacitated or requires hospitalisation
Neuro-control (ADL = activities of daily living)	no Rx. req., ADLs unaffected AND mild agitation or diff. concentrating or confusion	min Rx., some ADL limitation AND mod. confusion or agitation	Rx. req., needs ADL assistance AND severe agitation or confusion	toxic psychosis or hospitalisation
Muscle strength	subjective weakness no objective symptoms/signs	mild objective signs, symptoms, no dec. in function	objective weakness; function limited	paralysis

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
CARDIAC				
Cardiac Arrhythmia		Asymptomatic; transient dysrhythmia, no Rx required	Recurrent/persistent dysrhythmia; symptomatic Rx required	Unstable dysrhythmia, hospitalisation and Rx required
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; no Rx required	requires treatment
Hypertension	transient inc. >20 mm/Hg no Rx	recurrent; chronic increase > 20 mm/Hg Rx req.	acute Rx required; outpatient hosp. possible	requires hospitalisation
Hypotension	transient orthostatic hypotension; no Rx	symptoms correctable with oral fluid Rx	requires IV fluids no hosp. required	requires hospitalisation
Pericarditis	minimal effusion	mild/mod asymp. effusion, no Rx	symptomatic effusion, pain, ECG changes	tamponade OR pericardiocentesis OR surgery required
Haemorrhage, blood loss		mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss, >2 units transfused
GASTROINTESTINAL				
Stomatitis	mild discomfort, no limits on activity	some limits on eating/talking	eating/talking very limited	req. IV fluids
Nausea	mild OR transient; reasonable intake maintained	mod. discomfort; OR intake decreased for <3 days	severe discomfort OR minimal intake for >3 days	hospitalisation required
Vomiting	mild OR transient; 2-3 episodes per day OR mild vomiting lasting <1 week	mod OR persistent; 4-5 episodes per day; OR vomiting lasting >1 week	severe vomiting of all food/fluids in 24 hours OR orthostatic hypotension OR IV Rx required	hypotensive shock OR hospitalisation required

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
Oral Discomfort/ Dysphagia	mild discomfort, no difficulty swallowing	difficulty swallowing but able to eat and drink	unable to swallow solids	unable to drink fluids; IV fluids required
Constipation	mild	moderate	severe	distension with vomiting
Diarrhoea	mild OR transient; 3-4 loose stools per day OR mild diarrhoea lasting <1 week	mod OR persistent; 5-7 loose stools per day OR diarrhoea lasting >1 week	bloody diarrhoea; OR orthostatic hypotension OR >7 loose stools/day OR IV Rx required	hypotensive shock or hospitalisation required
OTHER PARAMETERS				
Fever; oral, >12 hours	37.7-38.5C or 100.0-101.5F	38.6-39.5C or 101.6-102.9F	39.6-40.5C or 103-105F	>40.5C or >105F
Headache	mild, no Rx required	mod or non-narcotic analgesia Rx	severe; OR responds to initial narcotic Rx	intractable; OR requiring repeated narcotic Rx
Fatigue	normal activity reduced <25%	normal activity reduced 25-50%	normal activity reduced >50%; can't work	unable to care for self
Allergic reaction	pruritus w/o rash	localised urticaria	generalised urticaria or angioedema	anaphylaxis
Cutaneous/Rash/ Dermatitis	erythema, pruritus	diffuse, maculopapular rash OR dry desquamation	vesiculation OR moist desquamation OR ulceration	ANY ONE: Mucous membrane involvement, suspected Stevens-Johnson (TEN), erythema multiforme, necrosis req surgery, exfoliative dermatitis

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
GUIDELINES FOR ESTIMATING GRADE OF SEVERITY FOR CONDITIONS NOT LISTED IN TABLE				
Criteria				
Karnofsky score	80-90	60-70	40-50	<40
or				
Self care ability, impact on activities of daily living (ADL)	Transient or mild discomfort; no limitation on activity	Mild to moderate impact on activity; may be able to work full-time; some assistance may be needed	Marked impact; ADLs limited; may work part-time with some assistance	Completely disabled, needs full assist with ADLs, unable to work
or				
Medical care needed	No therapy; monitor condition	May require minimal intervention and monitoring	Requires medical care and possible hospitalisation	Requires active medical intervention; hospitalisation or hospice care



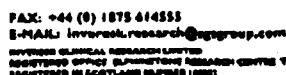
TRANENT EH33 2NE SCOTLAND
TELEPHONE: 044 (0) 1875 614343

ICR STUDY NO: 013177

**A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.**

PROTOCOL STATUS: AMENDMENT 1 – 3 NOVEMBER 1998

CONFIDENTIAL		DATE
Compiled by	K. Srinivas	27 Nov 98
Approved by	S. J. Srinivas	3 Nov 98
Copied by	S. J. Srinivas	5 Nov 98
Number of Copies Made	5	
Copies Distributed as Follows:-		
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5. N. Srinivasan (Team A File)	✓	04 Nov 98
6. DR / CRA (JALM)	✓	5 Nov 98
7. Pharmacy	✓	5 Nov 98
8. G. Srinivas	✓	03 Nov 98
9. Clinical Pathology	✓	5 Nov 98
10. Original Study (U)	✓	04 Nov 98
11.		



DIVISION CLINICAL RESEARCH LIMITED
 REGISTERED OFFICE: GLASGOW RESEARCH CENTRE TRINITY ONE ONE
 REGISTERED IN SCOTLAND NUMBER 10002

ICR 013177 – AMENDMENT 1 – 3 NOVEMBER 1998

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ICR 013177 – AMENDMENT 1 – 3 NOVEMBER 1998

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Persons authorised to sign protocol and amendments

Sponsor:	Dr C F Wilkinson
ICR:	Dr S Freestone
	Dr J Dickson

ICR 013177 - AMENDMENT 1 - 3 NOVEMBER 1998

I acknowledge possession of, and have read, a written summary of the pre-clinical and clinical data available on the compound to be studied in this protocol. I have had the opportunity to discuss the data and their implications with staff of the manufacturer. Having fully considered all the information available, I consider it is safe and ethically justifiable to give the compound to volunteers according to the agreed protocol.

Signed S J. Jones Date: 3 November 1998
Study Director

SIGNATURE PAGE

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.

THE UNDERSIGNED AGREE TO PERFORM THE STUDY ACCORDING TO THIS
PROTOCOL.

Study Director: S J. [signature] Date: 3 Nov 98

Clinical Investigator: [signature] Date: 3 NOV 98

Sponsor Representative: Chris F. Williams Date: Nov. 2, 1998

PROTOCOL CHANGES REQUESTED BY ETHICS COMMITTEE

Comments raised by the Ethics Committee were addressed in a letter by Dr S Freestone to the Chairman of the committee.

ADDITIONAL PROTOCOL CHANGES

Protocol

Page 2 of 69: Project Clinical Research Associate has been amended to Kay Whalley.

Page 3 of 69: Sponsor Medical Contact: Dr Anna-Mette Nielsen

Page 10 of 69: Urinary Pregnancy Test added to schedule of events in the safety section and the planned study dates have been amended.

Page 11 of 69: "in pharmacokinetic studies using radio-labelled malathion most...." Was added to the 3rd sentence in the second paragraph.

Page 12 of 69: 5th paragraph - Human Data: The paragraph now reads "A repeat dose oral study in volunteers (24 mg per person per day for 56 consecutive days) showed an equivocal reduction in plasma and RBC Cholinesterase after cessation of dosing. The study was of dubious quality and the effect observed probably artefactual."

Page 12 of 69: 6th paragraph: Inserted into the first sentence after 'figure' - (considered a safe daily human intake over a lifetime).

Page 13 of 69: First sentence: 'Major' amended to 'measurable', and 'and are very much lower than doses expected to cause any clinical signs' has been added to the end of the sentence.

Page 13 of 69: Section 4.1.1. Second sentence: The word 'studies' amended to 'studied'.

Page 14 of 69: 7th paragraph: The paragraph has been amended to read "In the final session, 7 females will receive the NOEL dose identified in males and 3 females will receive placebo. If a NOEL in males is not identified at doses up to and including 15 mg.kg⁻¹, further cohorts of men may be studied and dosing in females delayed until a NOEL is identified.

Page 16 of 69: Section 4.3.1. Pre-study screen. Point 2: Inserted after 'pulse rate' '(supine and erect)' and at the end of the sentence 'and oral temperature'. Point 7 has been added.

Page 17 of 69: Section 4.3.3. Exclusion Criteria: Point K amended to read "Smokers who cannot abstain from smoking from 2 hours predose to 8 hours postdose".

Page 18 of 69: Section 4.3.5. Second sentence: 'should' amended to 'will'.

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Page 18 of 69: Last sentence of 5th paragraph now reads "where subjects are withdrawn because of reasons 2 or 3 they will be replaced, if insufficient data is obtained".

Page 21 of 69, Section 4.5.1.1: Last sentence has been added to paragraph 1 and the 3rd paragraph deleted.

Page 26 of 69, Section 4.5.1.5.5: First sentence has been changed to "available for use by medical staff" as 2-PAM will be kept at Edinburgh Royal Infirmary and not within the clinical unit.

Page 27 of 69, Section 4.6, 4th paragraph: Last sentence in brackets has been deleted.

Page 28 and 29 of 69: Dr M McGuire amended to D.L. Scott

Page 28 of 69, Section 4.7.2: The specific alkyl phosphates have been added to the 6th paragraph dimethyl phosphate, dimethyl thiophosphate and dimethyl dithiophosphate
The urine volume to be collected has been increased to 2x20ml aliquots.

Page 30 of 69: BP and HR amended to Vital Signs and Temperature deleted.

Page 31 of 69, Section 4.8.3, Study Day: Sentence has been added "Subjects must refrain from smoking from 2 hours predose". Paragraph 4 "Subjects in the habit of smoking may do so after this timepoint" added to the end.

Page 37 of 69, Second and third paragraph - first sentence: '-30min' has been deleted after 'ie'.

Page 44 of 69, Section 11: Dates of August and October amended to October 1998 and February 1999 respectively.

Volunteer Information

Page 51 of 69, Point 12: Blood volume has been amended to 235 mls from 180 mls.

PROTOCOL CHANGES REQUESTED BY SPONSOR

In amendment 1 several changes have been made at the request of the sponsor. These changes are shown in bold type. The principal changes are:

Substitution of "trial compound" for "drug" or "study medication".

Deletion of "Adverse Drug Reaction" definition (p23).

Insertion of new Section 4.7.2 on Analysis of Plasma Samples.

Changes to the Volunteer Information.

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SUMMARY

ICR STUDY NUMBER/SPONSOR CODE	013177
TITLE:	A randomised double blind ascending single oral dose study with Malathion to determine the No Effect Level on plasma and RBC cholinesterase activity.
SPONSOR:	Chemnova Agro A/S
REGULATORY STATUS:	For EPA Submission
STUDY OBJECTIVES:	To determine the safety of Malathion and to establish a no effect level in healthy human male and female volunteers.
STUDY DESIGN:	Double blind, randomised, placebo controlled.
STUDY POPULATION: Number of volunteers:	48 (4 subjects per dose level (3 active and 1 placebo) for the first two dose levels and 10 subjects per dose level thereafter (7 active and 3 placebo)).
Age/Sex:	18-50y (38 male and 10 female).
Study specific requirements:	Healthy, weight 50-100kg within + 15% ideal body weight.
INVESTIGATIONAL PRODUCT: Formulation:	Malathion
Route of administration:	Oral
Dosage(s) per day:	Single dose, ascending doses of 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg ⁻¹ . Females studied at NOEL dose.
EVALUATION CRITERIA: Safety:	
Plasma and RBC Cholinesterase:	Screening, on Days -9, -7, -5, -2, -1, and -30 min predose, 1, 2, 4, 8, 12, 24 and 48h postdose and Days 4, 7 and 14.
Vital Signs:	Screening, Day-1, predose and 2h, 4h, 8h and 24h postdose.
12 Lead ECG:	Screening, -30min predose, 2h, 4h, 8h and 24h postdose.
Continuous single channel ECG monitoring:	-30min predose to 4h postdose.
Haematology/Clinical Chemistry:	Screening, predose (0) and 24h postdose.
Urinalysis:	Screening and 24h postdose.
Urine pregnancy test:	Screening and Day -1.
Physical Examination:	Screening and 48h postdose.
Adverse Events/Clinical Signs:	Throughout study.
Urine Drug Screen:	Screening.
Virology:	Screening.
Pharmacokinetics:	
Plasma:	Predose (0), 1, 2, 4, 8, 12, 24, 48 and 72h postdose
Urine:	Predose (-12-0h), 0-12h, 12-24h, 24-48h postdose
STUDY LOCATION:	
Clinical phase:	Inveresk Clinical Research
Clinical pathology:	Inveresk Research
Bioanalytical analysis:	Inveresk Research
Statistical analysis:	Inveresk Research
PLANNED STUDY DATES:	
Start of clinical phase:	October 1998
Completion of clinical phase:	February 1999
DURATION OF STUDY: (per subject)	14 days postdose

2. **BACKGROUND INFORMATION**

INTRODUCTION

Cheminova Agro A/S wishes to carry out an oral dosing study on malathion, an organophosphate insecticide that has been used for many years on a wide range of plants and crops in many parts of the world. It is also a constituent of lotions and shampoos used in the treatment of scabies and head lice. As a result of this use, accidental and incidental exposure of workers occur continually and the public may be exposed to residues of malathion in food, water and from residential and/or garden uses. The primary objective of the proposed study is to determine the highest dose of malathion causing no effect or lowest dose causing a slight inhibitory effect on blood cholinesterase in humans. This information will be used to provide a more accurate assessment of the margin of safety associated with currently estimated human exposures. The proposed study will investigate, under carefully controlled conditions, the effects of single oral doses of malathion on humans and will identify a dose level with no effect on plasma and RBC cholinesterase activity.

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION AND KINETICS

Malathion is rapidly and almost completely absorbed orally. Excretion is mainly in the urine, 80% as it's monocarboxylic and dicarboxylic acids which are inactive. In pharmacokinetic studies using radio-labelled malathion, most radio-label is removed from the body in 24h so there is little likelihood of accumulation of malathion or its metabolites.

TOXICOLOGY

The single dose toxicity of malathion is relatively low, with oral LD₅₀ values of at least 1000 mg.kg⁻¹. Its effects are due to inhibition of cholinesterase activity.

A 28 day dietary rat study indicated a No Observed Effect Level (NOEL) of 25 mg.kg⁻¹ based upon cholinesterase depression, and 28 day dog studies suggested cholinesterase depression at 125 mg.kg⁻¹.

A NOEL of approximately 5 mg.kg⁻¹ for cholinesterase inhibition was indicated in a chronic rat dietary study.

GENOTOXICITY AND CARCINOGENICITY

Ames tests were negative. Positive in-vivo chromosomal aberration results occurred in mice and hamsters but not rats.

A series of carcinogenicity studies indicated no clear carcinogenic activity. Any such potential seems to be slight and of little relevance to a single dose study.

HUMAN DATA

A repeat dose oral study in volunteers (24 mg per person per day for 56 consecutive days) showed an equivocal reduction in plasma and RBC Cholinesterase after cessation of dosing. The study was of dubious quality and the effect observed probably artifactual.

The Acceptable Daily Intake (ADI) figure (considered a safe daily human intake over a lifetime) suggested by the Pesticide Safety Division is 0.05 mg.kg⁻¹ day⁻¹ calculated using a 100-fold safety factor in the NOEL in a rat carcinogenicity study. The JMPR summary suggested 0.3 mg.kg⁻¹ as an ADI for man.

OVERVIEW

Malathion is rapidly absorbed after oral administration and almost entirely excreted within 24 hours.

It is of moderate to low toxicity with oral LD₅₀ values of 1000 mg.kg⁻¹ and greater. The main effect is inhibition of cholinesterase.

The proposed doses of 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹ are unlikely to have measurable effects on cholinesterase activity, and are very much lower than doses expected to cause any clinical signs.

3. STUDY OBJECTIVES

The study is to be undertaken in male human volunteers to establish an acute, single dose, oral No-Observed-Effect-Level (NOEL). This is defined as the highest dose tested at which no inhibition of plasma and red blood cell cholinesterase activity occurs. A group of female volunteers will be studied subsequently at the NOEL identified in males.

4. METHODS AND INVESTIGATIONAL PLAN

4.1 Study design and plan

4.1.1 Design of Study

Dose ranging covers 5 doses (0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹) and involves a maximum of 48 subjects (Table 1). If these doses do not result in inhibition of cholinesterase activity, higher doses may be studied after consultation with the Ethics Committee and issue of an amendment. The study is a double blind comparison of the test compound against placebo.

TABLE 1

	Placebo	0.5 mg.kg ⁻¹	1.5 mg.kg ⁻¹	5.0 mg.kg ⁻¹	10.0 mg.kg ⁻¹	15.0 mg.kg ⁻¹	NOEL dose in ♂
Session 1 Sub 001-004	1	3					
Session 2 Sub 005-008	1		3				
Session 3 Sub 009-018	3			7			
Session 4 Sub 019-022	1				3		
Session 5 Sub 023-031	2				4	3	
Session 6 Sub 032-038	3					4	
Session 7 (?) Sub 039-048	3						7

The study will be executed in 7 treatment blocks. The first block will comprise of 4 subjects. One subject will receive placebo and three subjects will receive the lowest dose of the test compound (0.5 mg.kg⁻¹).

The second treatment block will also consist of 4 subjects. One subject will receive placebo and 3 subjects will receive 1.5 mg.kg⁻¹ of the test compound.

Session 3 will consist will consist of 10 subjects. 7 subjects will receive 5.0 mg.kg⁻¹ of the test compound and 3 subjects will receive placebo.

Session 4 will consist of 4 subjects. One subject will receive placebo and 3 subjects will receive 10.0 mg.kg⁻¹

Session 5 will consist of 9 subjects. If no effect is seen at 10.0 mg.kg⁻¹ in session 4 a further 4 subjects will receive 10.0 mg.kg⁻¹, 3 subjects will receive 15.0 mg.kg⁻¹ and two will receive placebo.

Session 6 will consist of 7 subjects. If no effect is seen at 15.0 mg.kg⁻¹ in Session 5 a further 4 subjects will receive 15.0 mg.kg⁻¹ and 3 subjects will receive placebo.

In the final session, 7 females will receive the NOEL dose identified in males and 3 females will receive placebo. If a NOEL in males is not identified at doses up to and including 15 mg.kg⁻¹, further cohorts of men may be studied and dosing in females delayed until a NOEL is identified.

4.1.2 Primary and Secondary Endpoints

In the absence of any pre-defined hypothesis, it is not possible to define primary and secondary endpoints for this study. All safety measures (i.e. adverse events, vital signs, and laboratory parameters) will be examined for any dose related trends.

4.1.3 Justification of Dose and Design

The oral route has been chosen as the route of administration as it is a major potential route of exposure to the compound. The doses have been chosen

after consideration of data from observations in earlier animal and human studies

4.1.4 Criteria for Stopping Study

The study will be discontinued if any subject exhibits more than 25% inhibition of red blood cell ChE or plasma ChE at two consecutive timepoints, after completing that dose level or if a mean inhibition in any cohort of more than 15% is noted at two consecutive timepoints.

4.1.5 Criteria for Dose Escalation

Progression to the next higher dose level will be permitted only after full review of all safety data indicates that it is safe to do so.

Dose escalation will not occur if any subject shows any signs or symptoms of organophosphate toxicity or has $\geq 25\%$ inhibition from baseline of red cell ChE or plasma ChE at two consecutive timepoints without associated symptoms or signs or a mean inhibition in any cohort of $\geq 15\%$ at two consecutive timepoints is noted.

4.2 Informed Consent

Before admission to the study each volunteer will be informed of the nature and the risks of the study and written informed consent will be obtained from the volunteers (Appendix A). Each volunteer's general practitioner will be asked if they have any objections to their patient's participation before the start of the study (Appendix B). A copy of Appendix A (volunteer information) will be given to the GP.

4.3 Selection Of Study Population

Subjects will be healthy male and female volunteers selected from the panel of volunteers recruited by ICR. A total of 48 will complete the study.

Subjects will be screened for inclusion in the study, according to the criteria for inclusion up to 21 days before dosing.

4.3.1 Pre-study screen

The screening examination will consist of:-

1. Medical history.
2. Complete physical examination and vital signs (pulse rate [supine and erect], respiratory rate, blood pressure and oral temperature).
3. 12-lead ECG recording.
4. Haematology, clinical chemistry, plasma and RBC cholinesterase and urinalysis (Appendix C).
5. Hepatitis B: Hbs-Ag.
HIV infection: HIV antibody.
Hepatitis C: Hep C Ab.
6. Urine screening for drugs, including drugs of abuse (including cannabis).
7. Pregnancy test (last cohort of females)

4.3.2 Inclusion Criteria

- (a) Males and females 18 to 50 years of age.
- (b) No clinically important abnormal physical findings at the screening examination.
- (c) No clinically relevant abnormalities in the results of laboratory screening evaluation including plasma and RBC cholinesterase (Appendix C).
- (d) Normal ECG.
- (e) Normal arterial pressure (BP) and heart rate (HR). These will be measured after resting supine for 3 minutes. Normal BP is taken to be 100 to 150 mm Hg systolic and 50 to 90 mm Hg diastolic. Normal HR is taken to be 50 to 90 bpm. Erect heart rate will be measured after standing for one minute. Normal erect HR is taken to be 50 to 100 bpm.
- (f) Body weight between 50 and 100 kg and within +/-15% of ideal body weight as given in the table of Appendix D.
- (g) Able to communicate well with the 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.

4.3.3 Exclusion Criteria

- (a) Administration of any investigational test compound in the period 0 to 3 months before entry to the study (0 to 4 months if the previous investigational test compound was a new chemical entity).
- (b) A need for any medication during the period 0 to 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 to 12 weeks before entry to 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 are not taking adequate contraceptive precautions.
- (j) Females with a positive pregnancy test.
- (k) Smokers who cannot abstain from smoking from 2 hours predose to 8 hours post dose.
- (l) Any subject with a resting pulse of <45 bpm, a systolic BP of <100 mm Hg or a PR interval on ECG of >210 ms.
- (m) Any subject who has had exposure to anti-cholinesterases (including home pest control products) within one month of dosing.
- (n) All agricultural workers or pest control applicators.

4.3.4 Restrictions

No alcohol, caffeine or concomitant medications (apart from paracetamol or other medications used to treat adverse events) will be allowed during study.

4.3.5 Withdrawal Criteria

ICR will make every reasonable effort to complete the study. If a subject wishes to leave the study at any time, he/she will be permitted to do so.

Every reasonable effort will be made by ICR to complete a final assessment. ICR will advise the sponsor of the withdrawal of the subject from the study.

A subject may 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

Any subject discontinuing the study compound prematurely because of reasons 1 or 4 will be considered to have completed the study. Where subjects are withdrawn because of reasons 2 or 3 they may be replaced, if insufficient data is obtained.

4.3.5.1 Data to be collected on withdrawal

If a subject withdraws for non-medical reasons at short notice, the following procedure will be adopted:

- the medical risks of withdrawing from the study within 12 hours of dosing will be explained to the subject.
- a physical examination will be performed.
- the 24h postdose safety assessments (vital signs, ECG, haematology, clinical chemistry, and urinalysis) will be performed.
- subject should sign a "Discharge Against Medical Advice" form, if necessary.

4.4 Investigational Products

4.4.1 Product Description

The investigational product will be provided by the Sponsor.

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The matching placebo will be provided by ICR.

The active ingredient of the investigational product is malathion.

The investigational product will be provided as a clear liquid, in a glass bottle to be stored in the dark at ambient temperature (15-25°C).

The placebo will be provided as lactose.

The placebo and active capsules will be non discernible; matching each other in weight and presentation.

The formulation will be administered by the oral route.

Doses will be prepared by direct weighing of the bulk malathion liquid into a size zero hard gelatin capsule.

The target weight of malathion in each capsule will be based on the dose level and the body weight at screening of the individual volunteer.

Weighing of malathion into capsule shells will be undertaken on a maintained and calibrated electronic balance with a readability capability of 0.01 mg. Capsules will be filled to an accuracy of +/-5% of target dose weight for each volunteer.

The weights of malathion transferred to each capsule shell for each volunteer will be independently verified by the pharmacist responsible for undertaking the dose calculation.

Sponsor personnel or independent monitors acting for the sponsor may also verify the weights of malathion transferred to each capsule shell during the dispensing exercise, or alternatively blinded summaries of proposed weighing may be transmitted to the client for their approval prior to the dispensing exercise being undertaken. Sponsor personnel or independent monitors acting for the sponsor who have witnessed dose preparation will not be permitted to monitor Case Record Forms to protect the integrity of the study.

As a minimum the pharmacy will prepare 2 reference samples from each dispensing exercise representing duplicates of the doses prepared for the heaviest and lightest volunteers being dosed for each group.

4.4.2 Sponsor's Responsibilities

The sponsor will supply a quantity of malathion taken from a fully characterised batch of malathion in an appropriate container.

This will be supplied with a Certificate of Analysis and a statement of the expiry or re-test date and should be received at ICR at least 7 working days before the start of the study.

The sponsor will provide the investigational product packaged to prevent contamination or deterioration during transport and storage.

The sponsor will have determined acceptable storage temperatures, conditions and times to be observed for the investigational product and will inform ICR of these. If reconstitution is a requirement the sponsor will advise ICR of the procedures required.

4.4.3 ICR's Responsibilities

The Investigational product will be stored under the control of the ICR Pharmacist in a secure facility appropriate for the advised storage conditions.

Investigational products that require re-packaging or preparation for administration to humans will be handled by applying appropriate GMP principles and will be labelled by the ICR pharmacist according to current standard operating procedures.

4.4.4 Reconciliation

An accountability record of utilisation will be maintained for the investigational product.

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Unused investigational product will be disposed of, or returned in accordance with written instructions from the sponsor.

4.4.5 Blinding

This is a double blind, placebo controlled study. The allocation to active or placebo will be random and based on the randomisation code generated by the statistics department of Inveresk Research.

It will be the responsibility of Inveresk Research to ensure that blinding is kept during the study.

A copy of the randomisation code will be held by the ICR pharmacist who will prepare the doses and he/she will not disclose the contents of the code to any member of the study team (except in circumstances detailed under "Emergency Procedures")

4.4.5 Concomitant Therapy

No concomitant therapy with the exception of paracetamol or other medications deemed necessary to treat adverse events will be allowed during the study. Atropine sulphate and a cholinesterase reactivator (pralidoxime; 2-PAM) will be available for use in the highly unlikely event that cholinergic symptoms or signs are observed.

4.5 Measurement of Safety

4.5.1 Safety Variables

4.5.1.1 Vital Signs

Supine systolic and diastolic arterial pressure will be determined by sphygmomanometry. Standing and supine pulse rates will be determined by palpation. Respiratory rate will be determined manually and oral temperature will be determined using a mercury thermometer.

Measurements will be made at screening, day-1, predose (0) and 2, 4, 8 and 24 hours after dosing.

4.5.1.2 Electrocardiography (ECG)

A 12 lead ECG will be obtained at screening, -30min predose and 2, 4, 8 and 24h postdose. If a subject shows an apparently abnormal ECG at any stage, repeat tracings will be made with the abnormality followed to resolution and additional lead recordings taken as deemed relevant.

4.5.1.3 Laboratory Data (Haematology/Clinical Chemistry)

Laboratory tests will be performed at screening, predose (0) and 24h postdose. Blood samples for clinical chemistry and haematology will be collected in pre-heparinised tubes (5.0 ml) and in EDTA-coated tubes (3.0 ml) respectively. Analyses are shown in Appendix C.

Laboratory tests showing abnormal values for any subject will be repeated as often as deemed necessary by the clinical investigator until the test values return to accepted limits or until an explanation other than compound effect is given.

Any abnormality fulfilling the common toxicity criteria (Appendix E) will be treated as an Adverse Event.

4.5.1.4 Urinalysis

Urinalysis (Appendix C) will be performed at screening and 24h postdose. Any abnormality fulfilling the common toxicity criteria (Appendix E) will be treated as an Adverse Event.

4.5.1.5 Additional Safety Variables

Single Channel Continuous ECG Monitoring

Single channel continuous ECG monitoring will be performed using a bedside monitor from -30min predose to 4h postdose.

Any subject demonstrating sustained bradycardia ie. <50 bpm. or tachycardia i.e. >110 bpm for more than 30 seconds, will be reviewed by the clinical investigator.

4.5.1.5 Adverse Events

4.5.1.5.1 Definitions

Adverse Event

An adverse event is any unwanted event occurring during the course of a clinical trial.

Serious Adverse Events (SAE)

These are defined as adverse events which are fatal or considered life-threatening, which require hospitalisation or prolong hospitalisation, cause permanent disability, cancer, congenital anomaly or overdose, or are considered serious for any other reason.

Adverse events include any symptom, physical sign, syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen during the course of a clinical trial, after administration of trial compound, whether considered related or not.

Adverse events may be volunteered spontaneously by the subject, or be discovered as a result of general questioning by the investigator or by physical examination.

Questions will be phrased so that they do not "lead" the subject into giving information which is not valid. They must be recorded on the Adverse Events Form of the subject's Case Record Form.

As far as possible, each adverse event must also be described by its duration (start date, time and duration), its severity (Appendix E), an assessment of its cause (coexisting disease, concomitant medication, the test compound, or others), its relationship to the test compound (not related, unlikely, possibly, probably, definitely), and whether it required specific therapy.

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4.5.1.5.2 Qualification of Adverse Events

Severity

All adverse events must be rated on a 3-point scale of increasing severity (mild, moderate or severe).

4.5.1.5.3 Relationship to Trial Compound

The relationship to the trial compound of all adverse events will be categorised according to the following table:

Relationship to Trial Treatment or Test Compound

1	NOT RELATED	This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
2	UNLIKELY (must have two)	In general, this category can be considered applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test compound. An adverse event may be considered unlikely to be related if or when: 1) It does not follow a reasonable temporal sequence from administration of the test compound. 2) It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It does not follow a known pattern of response to the test compound.
3	POSSIBLY (must have two)	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test compound administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when: 1) It follows a reasonable temporal sequence from administration of the compound. 2) It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It follows a known pattern of response to the test compound.
4	PROBABLY (must have three)	This category applies to those adverse events which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test compound. An adverse event may be considered probably related if or when: 1) It follows a reasonable temporal sequence from administration of the compound. 2) It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the compound, yet compound-relatedness clearly exists (e.g., bone marrow depression, fixed compound eruptions, tardive dyskinesia). 4) It follows a known pattern of response to the test compound.
5	DEFINITELY (must have all)	This category applies to those adverse events which the investigator feels are incontrovertibly related to test compound. An adverse event may be assigned an attribution of definitely related if or when: 1) It follows a reasonable temporal sequence from administration of the compound. 2) It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3) It disappears or decreases on cessation or reduction in dose and recurs with re-exposure to compound. (Note: this is not to be construed as requiring re-exposure of the subject, however, a category of definitely related can only be used when a recurrence is observed). 4) It follows a known pattern of response to the test compound.

4.5.1.5.4 Notification

ICR will notify the ethics committee of all adverse events.

Any serious adverse event will be notified to the sponsor within 24 hours by telephone or fax. This will be followed up by a full written report within three days.

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The event must also be recorded on the standard Adverse Events Form, as described above.

These reports are to be made whether or not the investigator considers the serious adverse events to be related to the investigational compound.

A full written report will include photocopies of results, consultant report(s), and a summary of the outcome of the reaction plus the investigator's opinion of compound relationship to the serious adverse event(s).

4.5.1.5.5 Emergency Procedures

Emergency equipment and drugs (including atropine and 2-PAM) will be available for use by medical staff. In the unlikely event that they are required, their use will be documented.

Atropine antagonises the effects of accumulated acetylcholine and is the specific antidote for organophosphate toxicity. 2-PAM is a cholinesterase reactivator.

Additional supplies of atropine will be available at ICR for the duration of the study.

Atropine sulphate IV will be administered in increments of 0.6 mg up to a maximum of 3 mg by the clinical investigator in the event of severe symptoms/signs of organophosphate toxicity.

Atropine sulphate must be administered at the discretion of the clinical investigator in the following situations:-

- Symptomatic bradycardia (<50 bpm)
- Bradycardia (<50 bpm) associated with hypotension (systolic BP <90 mmHg).
- PR Interval >240 ms or evidence of heart block.
- Worsening of muscle fasciculation or evidence of respiratory depression.

Atropine sulphate administration should be considered if the following occur:-

- Moderate/severe nausea/vomiting/diarrhoea/abdominal cramps causing distress.
- Moderate/severe muscle or tongue fasciculation causing distress.
- Severe sweating or salivation causing distress.
- Any combination of the above.

Copies of the randomisation schedule will be held at Inveresk Research Regulatory Affairs Department in the project file and at the investigational site in sealed envelopes in study file 3. The Study Director may request that the envelope be opened in the event of an emergency.

4.5.1.5.6 Follow up of subjects experiencing adverse events.

Subjects who experience adverse events will be followed up until resolution or until a non compound related cause has been established.

4.6 Measurement of Effects

Cholinesterase assay of Blood Samples.

Samples will be collected during the course of the study at the following times:

Screening, Days -9, -7, -5, -2, -1 and -30min predose. All samples before dosing will be taken in the morning, at the same time of day if possible.

Blood (4.5 ml) will be collected into EDTA tubes from each subject and the tubes will be placed in ice.

After centrifugation the plasma and RBC fractions will be separated into 2 aliquots each. Samples up to 4h post dose will be transported to the lab at ERC on wet ice and assayed the same day. Samples later than 4h post dose will be centrifuged, separated and stored frozen overnight for assay on the next day.

The collected plasma and RBC samples will be assayed for cholinesterase (ChE) activity according to IR/SOP/CLC/636 and IR/SOP/CLC/920.

4.7 Collection and Analysis of Samples for Malathion Concentrations

4.7.1 Collection of Blood Samples

Samples will be collected for measurement of malathion and its malaoxon metabolite concentrations at the following timepoints:

Predose (0), 1, 2, 4, 8, 12, 24, 48 and 72h postdose

Blood (ca 14 ml) will be collected via a cannula or by repeated venepuncture into two 7 ml lithium heparin tubes. Plasma will be obtained by centrifugation. A minimum of 5 ml of plasma is required for analysis, therefore the plasma obtained from both blood tubes will be pooled as 1 plasma sample and stored at -70°C. Samples will be transferred frozen in dry ice to:

Dr D L Scott
Department of Bioanalytical Chemistry
Inveresk Research
Tranent
EH33 2NE
SCOTLAND

Analytical methods and other details of the analysis will be included in separate protocols.

4.7.2 Analysis of Plasma Samples

Analytical methods and other details of the analysis will be included in separate protocols.

4.7.3 Collection and Analysis of Urine Samples

Urine will be collected for measurement of malathion mono- and di-carboxylic acids and dimethyl phosphate, dimethyl thiophosphate and dimethyl dithiophosphate at the following times:

-12-0h (predose), 0-12, 12-24 and 24-48h postdose.

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Urine volume will be measured and 2 x 20ml aliquot from each period will be frozen at -70°C until analysis. Samples will be transferred frozen in dry ice to:

**Pacific Toxicology
6160 Varlet Avenue
Woodland Hills
CA 91367
USA**

Analytical methods and other details of the analysis will be included in separate protocols.

4.8 Practicalities

**4.8.1 Schedule of Events
(see overleaf)**

SCHEDULE OF EVENTS

	Pre-study	Predose		Times after dosing (h)											Post Study Days		
		Day -1	-30min	0	1	2	4	8	12	24	48	4 (i.e. +72h)	7	14			
Informed Consent		X															
History	X																
Physical Examination	X										X						
Compound Admin				X													
Haematology	X			X						X							
Clinical Chemistry	X			X						X							
Urinalysis	X									X							
ECG (12 lead)	X			X		X	X	X		X							
Vital Signs	X	X		X		X	X	X		X							
Urine drug screen	X																
Virology	X																
Pregnancy Test (♀ cohort)	X	X															
pk blood sample					X	X	X	X	X	X	X	X					
Cholinesterase Assay	X	X ¹	X		X	X	X	X	X	X	X	X	X				
Adverse Events																	
Test Compound Accountability																	
Continuous Single Channel ECG																	
Urine																	

¹ samples for cholinesterase assay will also be collected on Days -9, -7, -5 and Day -2.

4.8.2 Prestudy

Subjects will be admitted to the clinic on the morning before the study. The continued good health of each subject will be confirmed by enquiry and a brief examination (vital signs) on admission to the clinical unit. Blood samples will be obtained for plasma and red cell cholinesterase. A urine pregnancy test will be undertaken on female subjects.

A medication history update will also be obtained. Subjects will take no food or drink from 23.00h.

4.8.3 Study Day

Subjects must refrain from smoking from 2 hours predose.

At approximately 08.00h, subjects will be given a standard breakfast.

Approximately five minutes following completion of breakfast the formulation will be administered to subjects in the sitting position with 150 ml of water. The time of dosing will be recorded for each subject.

The subjects will remain seated or recumbent in the dosing area until approximately 8h postdose at which time they may be ambulant. Subjects in the habit of smoking may do so, after this timepoint.

Subjects will remain fasted until approximately 4h postdose when a light lunch will be taken. Water, fruit juice (not grapefruit juice) or decaffeinated drinks will be allowed on request from approximately 3h postdose.

Normal activities, but excluding strenuous exercise, will be permitted from 8h postdose.

Actual times of the main events will be recorded.

Subjects will not be permitted to take drugs on the study day or until after the last blood sample has been withdrawn on Day 14 and will be advised not to drink alcohol until after the visit at 72h post dose (i.e. Day 4).

4.8.4 Post-study

Before discharge (approximately 48h post dose) all subjects will have a physical examination. Any abnormalities in investigations will be followed to normality.

All subjects will attend for a post-study follow-up visit on the mornings of Days 4 (72h post dose), 7 and 14 to ensure continued well-being and completion of any outstanding enquiries/adverse events and for collection of a blood sample for measurement of malathion and metabolite (day 4 only) and plasma and RBC cholinesterase activity (days 4, 7 and 14).

4.8.5 Critical Phases

All critical phases of the study will be supervised by medical and nursing personnel. Any deviations from the protocol will be recorded. A physician should be present for at least 3h after dosing.

4.8.6 Conditions for Modifying or Terminating the Study

Protocol Amendments

All changes or revisions of this protocol will be documented, signed and dated by the Study Director. The reason for the amendment will be stated.

All amendments will be sent to the sponsor and the ethics committee for approval and will be retained to the original protocol.

4.8.7 Conduct of the Study

The study will be conducted in accordance with the guidelines set out in the Declaration of Helsinki, 1964, as amended by the 29th Medical World Assembly in Tokyo, 1975, the 35th Medical World Assembly in Venice, 1983, the 41st Medical World Assembly in Hong Kong, 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996.

The study will be undertaken on the understanding that it may be modified or abandoned at the sole discretion of the ICR clinical investigator after consultation with the sponsor.

4.8.8 Confidentiality

It is agreed that the information contained in this protocol and the results of the study will not be disclosed to others, without written authorisation from the sponsor, except to staff involved in the study.

5. STATISTICS AND DATA MANAGEMENT

5.1 Randomisation

A subject number will be assigned for all subjects who qualify for the study in accordance with exclusion and inclusion criteria. The number will be assigned using the lowest number available.

Subject numbers will be allocated according to the code 001-099 and replacement subjects will be identified by 901-999. Based upon a computer-generated randomisation, subjects will be assigned to one of the dose levels.

The randomisation code will be held by the Regulatory Affairs Department at Inveresk Research and the Pharmacy Department at ICR where it is required for dispensing purposes.

The blindness of the study will be broken on completion of the clinical phase and after all adverse events have been assigned (i.e. relationship to test compound determined) and coded.

Any request for issue of the code must be made in writing by the Study Director using a Randomisation Distribution Form. Two sets of sealed envelopes will be provided containing individual subjects codes and retained with the study filing at ICR. They may be used in case of an emergency by the supervising physician or after discussion with the sponsor. Any code break will be recorded and a copy kept with the study file.

The randomisation and disclosure envelopes will be produced by the Statistics and Data Management Department of Inveresk Research according to standard procedures.

5.2 Sample Size Justification

The sample size of 48 subjects (4 or 10 subjects per dose level) is considered appropriate for a study of this type. No formal sample size calculation was performed.

5.3 Data Recording

The Case Record Forms will be prepared by ICR and will be reviewed by the Statistics and Data Management Department of Inveresk Research.

All data obtained during the course of the clinical phase of the study will be recorded directly and legibly into the Case Record Form in black ink.

5.4 Data Management

Data management will be performed by the Statistics and Data Management Department at Inveresk Research.

Adverse events and medications will be coded using the WHO Adverse Reaction Terminology (1989 or a more recent version) and WHO Drug Reference List (1991 or a more recent version) respectively. Primary and secondary coding will be performed and discrepancies adjudicated by an independent third party.

All study data recorded in the Case Record Form (CRF), except clinical chemistry, haematology, RBC and plasma cholinesterase will be subjected to double data entry using a validated database programmed in a clinical data management system. On completion of data entry the data will be exported to SAS for further consistency and validation checks. Following comparison of the data entries, the database will be locked and the audit trail switched on ie a computerised log of all subsequent changes to the data will

be recorded. All data queries will be raised using Data Resolution Forms (DRFs) and will be resolved with the assistance of ICR medical staff.

On resolution of all data queries, the database will be closed and all study data will be exported to SAS (v6.07) for the production of data listings and summary tables.

Clinical chemistry, haematology, RBC and plasma cholinesterase data will be collected by the online clinical pathology system and electronically transferred to SAS for the production of summary tables and data listings.

Plasma test compound and metabolite concentration data will be doubly entered into ASCII files. The resulting datasets will be compared and the final dataset will be reformatted in SAS and subjected to a 100% check against the source data. Furthermore plasma test compound concentration data will be reformatted via SAS to an ASCII file suitable for importing into WinNonlin (v1.1 or a more recent version) (a pharmacokinetics modelling package). The reformatted data will be entered in hours and all pharmacokinetic parameters presented in terms of hours. Pharmacokinetic parameter values will be received in the form of WinNonlin ASCII file output. This file will be read into SAS.

Urine test compound and metabolite concentration data will be doubly entered into ASCII files. The resulting datasets will be compared and the final dataset will be reformatted in SAS and subjected to a 100% check against the source data.

All data listings for inclusion into the study report, except adverse events, will be subjected to 10% quality control checks against the CRFs. All adverse events listings and summary tables will be subjected to 100% quality control checks.

On issue of the final report, Inveresk Research standard SAS datasets, used for the purposes of reporting and analysis, will be transferred to the Sponsor, if required.

5.5 Statistical Methods

The statistical package SAS (v6.07 or a more recent version) will be used to produce all summary tables and data listings. The summary tables and data listings will be produced by the Statistics and Data Management Department at Inveresk Research.

Throughout this section it is assumed that data from the male placebo subjects from the different dosing cohorts will be combined.

In general terms, categorical data will be presented using counts and percentages, whilst continuous variables will be presented using the mean, standard deviation, median, minimum, maximum and number of subjects. In general, minima and maxima will be quoted to the number of decimal places as recorded in the CRF; means and standard deviations will be quoted to one further decimal place. Percentages will be rounded to one decimal place.

5.5.1 Demographics and Other Baseline Characteristics

The following demographic variables will be summarised by gender and dose level: race, age, height, weight, and physical examination (normal/abnormal by body system). No significance testing of demographic data will be performed.

5.5.2 Safety Parameters

The objective of the statistical analysis is to investigate the data for any effects of test material on clinical tolerability and laboratory safety parameters. All such parameters will be summarised by gender, dose level and timepoint. With the exception of RBC and plasma cholinesterase, no formal statistical analysis will be performed.

RBC and plasma cholinesterase will be summarised (ie mean, standard deviation, minimum, maximum and n) at each timepoint, including changes from baseline, by gender and dose level. Additionally, the percentage change from baseline at each timepoint will be tabulated by gender and dose

level and illustrated graphically. Baseline will be defined as the mean of all available predose values (ie days -9, -7, -5, -2, -1 and -30 minutes).

For the male data, percentage change from baseline for RBC cholinesterase and plasma cholinesterase will be analysed using a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (ie 1, 2, 4, 8, 12, 24, 48, 72h (day 4), day 7 and day 14) and dose level by timepoint interaction. Subject will be included as a random effect. At each timepoint separately, a test for linear trend with dose will be performed using a linear contrast. In addition, using the error variance from the ANOVA pairwise comparisons between placebo and each dose level will be carried out, at each timepoint, using Student's 't'-tests. At each timepoint, if the test for linear trend is significant at the 5% level then the pairwise comparisons at that timepoint will not be adjusted for multiple comparisons. If the test for linear trend is not significant at the 5% level, a Bonferroni adjustment will be applied to the pairwise comparisons at that timepoint (ie each comparison will be tested at the 1.7% significance level). Treatment group LSMeans (ie means adjusted for any imbalance in the model) will be presented together with the significance level of the 't'-tests and the test for linear trend. In addition, where a Bonferroni adjustment was applied, the significance level of the pairwise comparisons after adjustment will also be presented.

For the female data, percentage change from baseline for RBC cholinesterase and plasma cholinesterase will be using a repeated measures analysis of variance (ANOVA) including terms for dose level, timepoint (ie 1, 2, 4, 8, 12, 24, 48, 72h (day 4), day 7 and day 14) and dose level by timepoint interaction. Subject will be included as a random effect. Using the error variance from the ANOVA, a comparison between placebo and active group will be carried out, at each timepoint, using a Student's 't'-test. Treatment group LSMeans (ie means adjusted for any imbalance in the model) will be presented together with the significance level of the 't'-test.

Distributional assumptions underlying the statistical analyses will be assessed as follows: Normality will be examined using a Shapiro-Wilk test while homogeneity of variance will be assessed by plotting the residuals against the predicted values for the model. If there is significant non-normality which cannot be resolved by transforming the data, the data will be analysed

excluding outliers. However, if the omission of outliers has no effect on the conclusions, the results of the full dataset only will be reported.

Vital signs will be summarised (ie mean, standard deviation, minimum, maximum and n) at each timepoint, including changes from baseline (ie predose), by gender and dose level. Additionally, the number of subjects with 'substantial' increases or decreases in blood pressure (> 20 mmHg) and heart rate (> 15 bpm) will be tabulated.

Laboratory parameters will be summarised at each timepoint including changes from baseline (ie predose), by gender and dose level. In addition, abnormal values outside normal ranges will be flagged in the data listings.

Descriptive statistical methods will be used to summarise the following data types by dose level and, where appropriate, timepoint:

Demographic details

ECG

Urinalysis

Adverse events

Plasma concentrations of metabolite

Urine test compound and metabolite concentrations

Throughout the study, all adverse events either observed by medical staff or professional collaborators, or reported by the subject spontaneously or in response to a direct question, will be evaluated by the investigator and noted in the adverse event section of the CRF.

Adverse events will be coded using the WHO Adverse Reaction Terminology. Adverse events will be reported by primary body system and preferred term. In the tabulations, counting will be performed by subject and not event ie a subject reporting the same event more than once will have that event counted only once. All adverse events commencing prior to dosing with test compound will be excluded from the summary tables but will be fully listed.

A data listing of all information relating to adverse events will be provided. This data listing will include all events a subject experiences. The following tables will also be provided, by gender and dose level:

number and percentage of subjects with at least one adverse event;
number and percentage of subjects with serious adverse events;
number and percentage of subjects with test compound-related adverse events.

The following breakdown of adverse events will be provided, by gender and dose level:

by body system, preferred term and severity;
by body system, preferred term and relationship to test compound.

5.6 Pharmacokinetic Methods

A measured test compound concentration vs. time curve will be produced, in graphic and tabular form, for each subject on both linear/linear and log/linear scales. Mean test compound concentration vs time curves will also be presented for each dose level separately. Summary statistics (ie mean, standard deviation, minimum, maximum, n and coefficient of variation) will be calculated for plasma concentrations for each time point and each dose level.

Pharmacokinetic parameter values will be estimated using WinNonlin pharmacokinetic software (v1.1 or a more recent version). Unless otherwise agreed with the sponsor, a non-compartmental model will be used to generate parameter estimates. The following pharmacokinetic parameter estimates will be calculated:

C_{max}(obs) the observed maximum concentration of test compound in plasma measured in a subject after dosing, determined by direct inspection of the plasma test compound concentration vs. time data.

T_{max}(obs) the time at which C_{max}(obs) was apparent, determined by direct inspection of the plasma test compound concentration vs. time data.

Tlag(obs) Lag time; the time delay between dosing and onset of absorption for test compounds dosed via extravascular routes, if apparent.

AUC (0-t) the area under the plasma test compound concentration vs. time curve from time zero to 't' hours (where 't' = the time point for the last sample on the pharmacokinetic profile in which test compound was detected) calculated using the linear or log/linear trapezoidal method.

AUC(0-∞) the area under the plasma test compound concentration vs. time curve from time zero to infinity: $[AUC(0-∞) = AUC(0-t) + (C_t/K_{el})]$, where C_t = the concentration of test compound for the last sample on the pharmacokinetic profile in which test compound was detected, and K_{el} = the terminal elimination rate constant, determined from the slope of the terminal elimination phase].

CL/F clearance: the apparent volume of the central compartment cleared of test compound per unit time after i.v. dosing (or per unit time and per unit of body weight if dosing data is entered as, for example, mg.kg⁻¹). The estimate does not account for the bioavailability (F, as a fraction of 1) and is therefore nominally divided by this value when test compound is given via extravascular routes.

T_{1/2el} terminal elimination phase half-life

Summary statistics (ie mean, standard deviation, median, minimum, maximum, n) and will be presented for all pharmacokinetic parameters by dose level. In addition, geometric mean and coefficient of variation (based on the logarithmically transformed data) will be presented for AUC and C_{max}(obs) by dose level. The coefficient of variation will be calculated using the following formula:

$$CV(\%) = [\exp(sd^2) - 1]^{1/2} \cdot 100$$

where sd = standard deviation of the logarithmically transformed data.

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AUC(0-∞) values will be analysed for dose proportionality using analysis of variance techniques (ref. Gough et al). The following model will be fitted:

$$\log(\text{AUC}) = \mu + \beta \cdot \log(\text{Dose})$$

This is usually referred to as a power model because after exponentiation:

$$\text{AUC} = \alpha \cdot \text{Dose}^\beta$$

The estimate obtained for β is a measure of dose proportionality. Dose proportionality requires that $\beta=1$ (if $\beta = 0$ this implies dose-independent parameters). The estimate of β together with its 95% confidence interval (β_L, β_U) will be presented to quantify the degree of non-proportionality.

The increase in AUC(0-∞) for a two-fold increase in dose will be calculated as 2^β . The confidence interval for this ratio will be obtained by substituting β_L and β_U in the equation.

The assumption of a linear relationship between the log AUC(0-∞) and log dose will be tested using analysis of variance by partitioning the sums of squares for treatments into those for linearity and departures from linearity. If the departures from linearity are significant then the test for dose proportionality will not be performed.

The individual AUC values will be presented graphically by dose level, in addition the linear regression line fitted will be displayed. The mean AUC values along with the standard deviation from the mean will also be displayed graphically by dose level.

Reference: Gough K., Hutchison M., Keene O., Byrom B., Ellis S., Lacey L., and McKellar J. Assessment of Dose Proportionality: Report from the Statisticians in the Pharmaceutical Industry/Pharmacokinetics UK Joint Working Party. Drug Information Journal, Vol 29, pp1039-1048, 1995.

6. DOCUMENTATION

6.1 Reporting

6.1.1 Interim Reports

Interim reports detailing adverse events and other relevant safety data, including cholinesterase results will be provided after each treatment block. These reports will be unaudited.

6.1.2 Study Reports

ICR will prepare the final report which will contain details of all safety data. The format of the report will be that of the Sponsor. The sponsor will provide a copy of his SOP for use by ICR within six weeks.

After the completion of the experimental work a draft report will be despatched to the sponsor.

6.1.3 Case Record Forms (CRFs)

CRFs will be prepared in two part NCR paper

Upon completion of the experimental work the top copy will be sent to the sponsor.

6.2 Archives

All data produced will be stored in the archives of Inveresk Research. All data will be stored for fifteen years but after 5 years the sponsor will be consulted regarding the continued storage of raw data which will be at additional cost to the sponsor

Samples that are unstable may be disposed of before fifteen years after consultation with the sponsor.

6.3 Data Protection

When personal data on subjects are stored or processed by computer, the data must be protected to prevent their disclosure to unauthorised third parties. The pertinent sections of the UK data protection laws will be complied with in full.

7. ETHICS REVIEW PROCEDURE

The protocol and details of the study will be submitted to the ethics review committee of Inveresk Research in advance of the study. The participation by the sponsor in the preparation of material for submission is required by ICR. The study will not proceed until the approval of the ethics review committee has been received.

The written approval of the committee will be retained as part of the study file.

All amendments will be submitted to the Ethics Committee Chairman for approval.

8. GOOD CLINICAL RESEARCH PRACTICE

This study will be conducted in accordance with the Guideline for Good Clinical Practice (CPMP/ICH/135/95).

9. GOOD LABORATORY PRACTICE

This work as described in protocol amendment 1 will be conducted in compliance with OECD principles of Good Laboratory Practice as set forth by the United Kingdom Department of Health and as accepted by Regulatory Authorities throughout the European Community, United States of America (FDA and EPA) and Japan (MHW, MAFF and MITI). All routine activities conducted during the course of this study are detailed in Inveresk Research's Standard Operating Procedures.

10. **QUALITY ASSURANCE**

Quality assurance inspections will be carried out during the conduct of the study by the Inveresk QA unit. Phases selected for inspection will include (but will not be limited to):

Pharmacy data review

Dose preparation

Dosing

On study observations

Review of on-study case record forms and associated data

Data entry, where appropriate

Statistical analysis

Clinical Pathology

Bioanalytical Chemistry

Both the draft report and the final report will be audited

Selection of subjects for audit will be at the discretion of the QA unit and may be varied at any time during the course of the audit. QA also reserve the right to conduct for cause inspection of any aspect of the study without prior notification.

Records of these inspections and audits will be documented and distributed to study management for review. Copies of internal Inveresk and any 3rd party QA consultant audit and inspection reports, with responses, will be provided by the Sponsor Representative and QA unit upon completion of each audit.

The conduct of these inspections and audits will be carried out according to standard operating procedures by quality assurance personnel of Inveresk Research, who are independent of those responsible for the trial.

11. **SPECIAL CONDITIONS**

The study will proceed only after documented acceptance of the protocol by the sponsor has been received by ICR.

12. TIMEPLAN

Study start:	October 1998.
Clinical phase completed:	February 1999.
Issue of draft report:	TBC
Issue of final report:	TBC

APPENDIX A

Volunteer Consent Form

This agreement is between the volunteer _____ and Inveresk Clinical Research Limited (hereinafter referred to as ICR), and provides for the volunteer to take part in experiments, trial and/or tests of a chemical compound or compounds.

FOR ALL STUDIES

- 1) I, the undersigned voluntarily agree to take part in

Protocol No: 013177

Descriptive Study Title: A randomised double blind ascending single oral dose study with malathion to determine the no effect level on plasma and RBC cholinesterase activity.

I understand that the investigation will involve the administration of

Name(s) of compounds: Malathion .

being the compound under test.

- 2) I have been given a full explanation by Dr _____ of the nature, purpose and likely duration of the study and what I will be expected to do and I have been advised about any discomfort and possible ill-effects on my health or well-being which he/she believes may result. The information document given to me is attached (Appendix A pages 50 of 70 to 53 of 70).
- 3) I have been given the opportunity to question Dr _____ on aspects of the study and have understood the advice and information given as a result.
- 4) I agree to Dr Freestone contacting my general practitioner (and teaching or university authority if appropriate) to make known my participation in the study and I authorise my general practitioner to report details of my relevant medical or drug history, in confidence.

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APPENDIX A (Continued)

- 5) I agree to comply with any instruction given during the study and to cooperate faithfully with Dr Freestone and to tell him immediately if I suffer from any deterioration of any kind in my health or well-being or any unexpected or unusual symptoms however they may have arisen.
- 6) I agree that I will not seek to restrict the use to which the results of the study may be put and, in particular, I accept that they may be disclosed to regulatory authorities for compounds in the UK and elsewhere.
- 7) I understand that I am free to withdraw from the study at any time without needing to justify my decision.
- 8) The supervising doctor confirms that subject to overriding requirement of law necessitating the disclosure of documents relating to the study, the volunteer will not be referred to by name in any document concerning the study disclosed to any person not under the direct control of the supervising doctor;
- 9) ICR confirms that:
 - (i) I shall receive in consideration for completing the study, the sum of £450 from the supervising doctor and that I shall receive the sum in full if it is necessary for me to withdraw from the study for medical reasons associated with participation in it. If I withdraw from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if I withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising doctor;

APPENDIX A (Continued)

- (ii) In the event of my suffering any bodily injury caused directly by my participation in the study, I may elect to proceed in accordance with the following optional procedure.
- (a) Compensation will be paid to me by ICR without my having to prove that the injury arose through negligence or that the study compound was defective as set forth in the Association of the British Pharmaceutical Industry "Guidelines for medical experiments in non-patient human volunteers".
- (b) The amount of such compensation shall be calculated by reference to the amount of damages commonly awarded for similar injuries by an English court if liability is admitted, providing that such compensation may be reduced to the extent that I, by reason of contributory fault through my actions or my failure to act, am partly responsible for the injury.
- (c) Any dispute or agreement as to the application of clause 9(ii) (a) and (b) may at my option be referred to an arbitrator to be agreed between myself and ICR, or in the absence of agreement, to be appointed by the President of the Royal College of Physicians of London with power in the arbitrator to consult a barrister of 10 years standing in respect of any issue of law including the amount of damages to be awarded as payment of compensation;
- (d) The agreement shall be construed in accordance with English law and subject to clause 9(ii) (a), (b) and (c) above the English courts shall have sole jurisdiction over any dispute which may arise out of it.

APPENDIX A (Continued)

Signed by the volunteer :

Dated :

Signed for on behalf
of ICR by its duly
authorised
representative :

Dated :

I confirm that I
have explained the
nature, purpose and
possible hazards of
the above trial to :

Signed :

Dated :

I confirm that I have witnessed the above explanation :

Signed :
Witness Signature

Dated :

(NB-It may be appropriate for the supervising doctor to fulfil the obligations of the
duly authorised representative for ICR.

Since signing the consent, the volunteer information sheet has been changed. I confirm
that I have read the revised information (dated / /) and still consent to participation in
the study.

Signed :

Dated :

APPENDIX A (Continued)

Volunteer information

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND RBC CHOLINESTERASE ACTIVITY.

Introduction

Malathion is an organophosphorus (OP) insecticide, one of a family of cholinesterase inhibitor compounds that includes many widely used insecticides. These pesticides have essentially no effects on mammals at sufficiently low levels. At higher levels they reduce the amount of an enzyme (cholinesterase) that breaks down a chemical substance (acetylcholine) in the body that is responsible for the transmission of nerve impulses, with the effect increasing as the dose increases. At yet higher levels, sufficient to reduce considerably the levels of acetylcholine in the nervous system, mild to serious physical effects can result (see the paragraph entitled Side Effects, below). Of all the OP insecticides, malathion has been shown to be among the lowest in acute toxicity (toxicity from a single dose) in animals and humans.

Very small amounts of residues of malathion may exist at harvest on treated crops. To determine whether these residues pose any risk to food consumers, many toxicity studies in animals, and one human study, have already been conducted. The animal studies showed that single doses at levels well above those that will be involved in this study produced no reduction in cholinesterase levels and no toxic effects.

Although the mechanism by which OPs act to inhibit cholinesterase is well understood, differences in behaviour of compounds from species to species do exist. This study is being conducted to reduce the uncertainties of species differences in determining a level of human exposure that causes measured reduction of blood cholinesterase levels. Prior testing has shown that a relatively large reduction of blood cholinesterase is required before any resulting clinical effects are observed. The results of this study are expected to be useful in showing that the use of malathion on crops does not pose health risks to food consumers, and also may be useful in evaluating whether workers who use malathion are thereby at risk.

Aim

The aim of the study is to determine the highest dose at which no significant reduction of blood cholinesterase occurs.

Dose

The doses to be given are 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹ body weight and placebo (inactive compound). A maximum of 48 of you will be tested in 7 groups. In the first group, one subject will receive placebo and three subjects the lowest dose of active compound (0.5 mg.kg⁻¹). In the second group, one subject will receive placebo and three subjects will receive 1.5 mg.kg⁻¹. In the third group three subjects will receive placebo and seven subjects will receive 5.0 mg.kg⁻¹. In the fourth group, one subject will receive placebo and three subjects will receive 10.0 mg.kg⁻¹. In the fifth group two subjects will receive placebo, four subjects will receive 10.0 mg.kg⁻¹ and three subjects will receive 15.0 mg.kg⁻¹. In the sixth group three subjects will receive

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APPENDIX A (Continued)

Volunteer Information

placebo and four subjects will receive 15.0 mg.kg⁻¹. When the maximum dose that causes no significant inhibition of blood cholinesterase in men has been identified, this dose will be given to a group of 10 women (7 to receive active compound and 3 placebo).

Allocation to receive active compound or placebo (inactive compound) will be randomised. The compound or placebo will be administered as capsules by mouth.

Side Effects

In view of the low doses of malathion to be used in this study and the results of earlier studies, it is not anticipated that any of the subjects of the study will experience any adverse effects other than a reduced level of blood cholinesterase. However, human studies with other more potent organophosphorus compounds suggest that the following effects can result from sufficiently high doses. Initial symptoms typically are headache and nausea, followed by a feeling of chest tightness and coughing. At dose levels much higher than those being used in this study other possible symptoms include vomiting, diarrhoea, abdominal pain, blurred vision, weakness, sweating, constricted pupils, excessive saliva production, slow pulse, and involuntary muscle twitching or spasm. A few reported cases of coma or death from ingestion of large quantities of malathion have been reported. However, the doses that cause these effects are very much higher than the ones to be used in this study. In some animal studies (but not all) it has been shown that lifetime exposure to 1600 mg/kg daily of malathion (ie more than 100 times the maximum dose in this study every day for a lifetime) in sensitive species caused an increased incidence of liver cancer. The dose level and duration of treatment bears no relationship to the single low doses to which volunteers will be exposed in this study.

Procedure

You will attend ICR for screening within 21 days of the start of the study. At screening, a complete medical history will be taken and you will have a complete physical examination, recordings of your pulse and blood pressure obtained, an ECG recorded (tracing of your heart's electrical activity) and blood samples taken for various safety tests. Samples of blood will also be taken to test for hepatitis B, hepatitis C and HIV, the virus that can cause AIDS. Urine will also be tested including a test for drugs of abuse.

Your GP will be informed of your participation and asked to confirm your medical history. If there are any objections expressed by your GP you will be excluded from the study.

Once you have successfully passed the screening examination, including acceptable blood and urine test results, your co-operation with the following will be required:

1. Up to a total of 48 of you will be studied.
2. If you smoke you must be able to abstain from smoking from 2h predose to 8h postdose.

APPENDIX A (Continued)

Volunteer information

3. You will require to attend the clinic for 4 outpatient visits on 9, 7, 5 and 2 days prior to dosing when a blood sample will be taken.
4. You will then be resident in the clinic on one occasion for 3 nights and will then return for 3 further outpatient visits 3, 6 and 13 days after dosing.
5. You will be admitted to the clinic between 10am and mid-day on the morning preceding the day of dosing. A brief examination including vital signs will be performed, and a blood sample taken for measurement of cholinesterase. A urine pregnancy test will be undertaken on female subjects. You will take no food or drink from 2300h.
6. On the day of dosing, you will be given breakfast and 5 min after completion you will receive either the active compound or placebo with 150 ml of water in the sitting position. You will then be required to remain seated or recumbent until 8h after dosing. You will be allowed water, fruit juice or decaffeinated drinks from approximately 3h after dosing. A light lunch will be provided approximately 4h after dosing. Normal activities excluding strenuous exercise will be allowed from approximately 4h after dosing.
7. Before dosing, a cannula (plastic tube inserted by a needle into a vein) will be inserted into your arm in order to allow samples of your blood to be obtained at regular intervals throughout the day of dosing. A needle and syringe can be used repeatedly to obtain blood samples if preferred. Repeated blood tests and cannulae can cause soreness and bruising of the arms or even, rarely, blockage of a vein, but those problems usually clear up within a few days to a few weeks.
8. Blood pressure, heart rate and a tracing of your heart's electrical activity will be recorded at intervals before and after dosing.
9. You will also be monitored for the presence or absence of the clinical signs listed in the introduction. A continuous recording of your heart's electrical activity will be displayed on a bedside monitor from 30mins before dosing to 4h after dosing.
10. You will be discharged 48h after dosing. Before discharge, a physical examination will be performed and blood samples will be taken for safety assessments.
11. You will return to the clinic in the morning 3, 6 and 13 days after dosing when a blood sample will be taken and to ensure continued well-being and for completion of any outstanding enquiry/adverse events.
12. Approximately 235 ml of blood will be taken during the study (compared with 480ml which is a standard blood donation).
13. You should avoid medication (including over-the-counter products) for 5 days before the start of the study.
14. You will not be allowed to take alcohol or other drugs on each resident study day or until after the last blood sample has been taken. It is recommended that you should refrain from alcohol as far as possible until the follow-up visit on Day 14.

APPENDIX A (Continued)

Volunteer information

If anything abnormal occurs, judged by the supervising clinician, or if laboratory investigations change, you may be withdrawn from the study. In addition, you may withdraw at any time without needing to justify your decision. (You are strongly advised not to leave the clinical unit within 24h after dosing as this may involve risk to your health). If you decide to withdraw before completion of the study a medical examination including blood pressure and heart rate, blood sampling, urine sampling and an ECG will be performed.

You should inform the supervising physician of any symptoms. After the study is over, you will be given a telephone number to call if you have any questions or worries.

It is essential that you should adhere to all of these requirements. The supervising physician will be pleased to supply any further information at any time.

All information will be treated in a confidential manner but anonymised data will be seen by authorised persons involved in the study and possibly by compound regulating authorities.

Supervising Physicians

Dr J Dickson and Dr S Freestone
Inveresk Clinical Research
Riccarton
Edinburgh
EH14 4AP Tel: (0131) 451 5080

APPENDIX B

Letter to Volunteer's General Practitioner

**STEPHEN FREESTONE MD, FRCPEd in
MEDICAL DIRECTOR**

Name and Address of Doctor

Date:

Dear Dr

Human Volunteer Study: A Randomised Double Blind Ascending Single Oral Dose Study with malathion to determine the No Effect Level on Plasma and RBC Cholinesterase activity.

Dates of Study:

Your Patient:

D.O.B.:

Inveresk Clinical Research Limited carries out compound evaluation and research as a preparation for regulatory agency submissions and product licence applications in the UK and other countries of the world. As part of its programme, volunteers help from time to time with the investigations.

We have invited your patient to take part in the above experimental study which will be carried out under full medical supervision at the Clinical Unit of Inveresk Clinical Research. He has consented to my contacting you. I enclose a copy of the signed volunteer consent form.

A brief statement of the nature of the test compound is contained in the attached summary.

If you have any medical objections to your patient taking part in this study, we would be grateful if you could inform us of your objections as soon as possible. In any event, it would be most helpful to us if you could complete and return the enclosed questionnaire.

Please address any account you may wish to send to Inveresk Clinical Research Limited. Fees should be in accordance with those recommended by BMA guidelines. Thank you in anticipation for your co-operation.

Yours sincerely

DR S FREESTONE

APPENDIX B (Continued)

STEPHEN FREESTONE MD, FRCPedin
MEDICAL DIRECTOR

Date:

Study No: 013177

Patient's Name:

Date of Birth:

General Practitioner:

MEDICAL HISTORY

YES NO
(Tick as Appropriate)

Disorders of the central nervous system:	<input type="checkbox"/>	<input type="checkbox"/>
Disorders of the cardiovascular system:	<input type="checkbox"/>	<input type="checkbox"/>
Disorders of the respiratory system:	<input type="checkbox"/>	<input type="checkbox"/>
Diseases and disorders of the alimentary system:	<input type="checkbox"/>	<input type="checkbox"/>
Disorders of the genito-urinary system:	<input type="checkbox"/>	<input type="checkbox"/>
Psychiatric disorders:	<input type="checkbox"/>	<input type="checkbox"/>
Alcohol/drug abuse:	<input type="checkbox"/>	<input type="checkbox"/>
Other (e.g. diabetes mellitus, thyroid disorder, allergic):	<input type="checkbox"/>	<input type="checkbox"/>
Recent prescribed drug treatment:	<input type="checkbox"/>	<input type="checkbox"/>
Any evidence of adverse drug reaction:	<input type="checkbox"/>	<input type="checkbox"/>
Do you have any objections to your patient participating in this trial?	<input type="checkbox"/>	<input type="checkbox"/>

If Yes, please specify

Has your patient participated in a trial of an unlicensed drug in the last 6 months?

Yes ☐ No ☐ Don't Know ☐

How long do your records cover the medical history of this patient? ____ Years

Signed: _____ Date: _____
General Practitioner

Authorisation Stamp: _____

Please note: This statement does not imply your consent. Responsibility for a subject entering a study is between the volunteer and ICR.

FOR OFFICIAL USE ONLY

Comments:	Signature:	Date of Review:

APPENDIX B (Continued)

STEPHEN FREESTONE MD, FRCPed in
MEDICAL DIRECTOR

Date:
Patient's Name:
Date of Birth:
General Practitioner:

Study No: 013177

MEDICAL HISTORY

A full questionnaire has been completed on the above patient within the last twelve months.

Has there been a significant change in this patient's medical history since _____?

Yes ☐ No ☐ (Tick as appropriate)

If yes, please comment: _____

Do you have any objections to your patient participating in this trial?

Yes ☐ No ☐ (Tick as appropriate)

If Yes, please specify: _____

Has your patient participated in a trial of an unlicensed drug in the last 6 months?

Yes ☐ No ☐ Don't Know ☐

How long do your records cover the medical history of this patient? _____ years

Signed: _____ Date: _____

General Practitioner

Authorisation Stamp: _____

Please note: This statement does not imply your consent. Responsibility for a subject entering a study is between the volunteer and ICR.

FOR OFFICIAL USE ONLY

Comments:	Signature:	Date of Review:
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AGE RANGE 18-50**APPENDIX C****Methods, Abbreviations and Units in Laboratory Investigations****Haematology**

<u>Parameter</u>	<u>Method</u>	<u>Normal Range</u>	<u>Units</u>
Haemoglobin: (Hb)	Technicon H1 Analyser Bayer UK Ltd	12.8 - 16.3 ♂ 10.8 - 15.3 ♀	g/dl
Total Red Blood Cell Count: (RBC)	Technicon H1 Analyser Bayer UK Ltd	4.17 - 5.55 ♂ 3.60 - 5.06 ♀	$\times 10^{12}/l$
Haematocrit: (Hct)	Technicon H1 Analyser Bayer UK Ltd	0.382 - 0.487 ♂ 0.321 - 0.457 ♀	l/l
Mean Cell Haemoglobin: (MCH)	Technicon H1 Analyser Bayer UK Ltd	27.4 - 32.3 ♂ 27.5 - 32.2 ♀	pg
Mean Cell Volume: (MCV)	Technicon H1 Analyser Bayer UK Ltd	81.4 - 96.3 ♂ 82.0 - 97.8 ♀	fl
Mean Cell Haemoglobin Concentration: (MCHC)	Technicon H1 Analyser Bayer UK Ltd	31.9 - 35.6 ♂ 31.6 - 34.9 ♀	g/dl
White Blood Cell Count: (WBC)	Technicon H1 Analyser Bayer UK Ltd	3.62 - 10.49 ♂ 3.84 - 10.82 ♀	$\times 10^9/l$

AGE RANGE 18-50APPENDIX CMethods, Abbreviations and Units in Laboratory InvestigationsHaematology

<u>Parameter</u>	<u>Method</u>	<u>Normal Range</u>	<u>Units</u>
Differential White Cell Count			
Neutrophils: (Neut)	Technicon H1 Analyser Bayer UK Ltd	1.58 - 7.47 ♂ 1.86 - 7.43 ♀	x10 ⁹ /l
Lymphocytes: (Lymp)	Technicon H1 Analyser Bayer UK Ltd	1.01 - 3.03 ♂ 1.09 - 3.16 ♀	x10 ⁹ /l
Monocytes: (Mono)	Technicon H1 Analyser Bayer UK Ltd	0.18 - 0.75 ♂ 0.16 - 0.67 ♀	x10 ⁹ /l
Eosinophils: (Eos)	Technicon H1 Analyser Bayer UK Ltd	0.04 - 0.47 ♂ 0.03 - 0.48 ♀	x10 ⁹ /l
Basophils: (Baso)	Technicon H1 Analyser Bayer UK Ltd	0.02 - 0.09 ♂ 0.02 - 0.11 ♀	x10 ⁹ /l
Large Unclassified Cells (LUC)	Technicon H1 Analyser Bayer UK Ltd	0.07 - 0.32 ♂ 0.09 - 0.36 ♀	x10 ⁹ /l
Platelets: (Plat)	Technicon H1 Analyser Bayer UK Ltd	128 - 331 ♂ 108 - 362 ♀	x10 ⁹ /l

AGE RANGE 18-50APPENDIX CMethods, Abbreviations and Units in Laboratory InvestigationsClinical Chemistry

<u>Parameter</u>	<u>Method</u>	<u>Normal Range d/s</u>	<u>Units</u>
Urea	Sampson E J <i>et al</i> (1980) Clin Chem <u>26</u> 816 - 826	1.7 - 8.3	mmol/l
Glucose: (Glu)	Anon (1983) J Clin Chem Clin Biochem <u>21</u> 749 - 760	3.8 - 6.5	mmol/l
Aspartate Amino- Transferase: (AST)	Bergmeyer, H.U <i>et al</i> , (1986) J Clin Chem Clin Biochem <u>24</u> 497 - 510	5 - 43	iu/l
Alanine Amino- Transferase: (ALT)	Bergmeyer, H.U <i>et al</i> , (1986) J Clin Chem Clin Biochem <u>24</u> 497 - 510	8 - 45	iu/l
Lactate Dehydrogenase: (LDH)	Bergmeyer H U (1975) Z Clin Chem Biochem <u>13</u> 507	229 - 460	iu/l
Sodium: (Na)	Tietz N (1986) Text Book of Clinical Chemistry, WB Saunders Company	135- 150	mmol/l
Potassium: (K)	Tietz N (1986) Text Book of Clinical Chemistry, WB Saunders Company	3.3 - 4.8	mmol/l
Chloride: (Cl)	Tietz N (1986) Text Book of Clinical Chemistry, WB Saunders Company	97 - 109	mmol/l
Total Protein: (TP)	Friedman R <i>et al</i> (1980) Clin Chem <u>26</u> 4	66 - 87	g/l

AGE RANGE 18 - 50APPENDIX CMethods, Abbreviations and Units in Laboratory InvestigationsClinical Chemistry

<u>Parameter</u>	<u>Method</u>	<u>Normal Range c/l</u>	<u>Units</u>
Albumin: (Alb)	Friedman R et al (1989) Effects of Disease on Clinical Laboratory Tests, AACC Press, Washington DC	41 - 54	g/l
Creatinine: (Crea)	Butler A R (1975) Clin. Chem. Acta. <u>59</u> 227-232	77-136	μmol.l ⁻¹
T.Bilirubin: (T.Bil)	Henry R J et al (1974) Clin Chem Principles and Techniques, Harper and Row 1059	3.7 - 21.0	umol/l
Gamma Glutamyl Transpeptidase: (GGT)	Young D S et al (1975) Clin Chem <u>21</u> 5	3 - 60	iu/l
Plasma Cholinesterase (Plasma ChE)	Ellman, G.L., et al, Biochem Pharmacol, 7:78, (1961)	3000-8000	iu.l ⁻¹
Red Cell Cholinesterase (RBC ChE)	Ellman, G.L., et al, Biochem Pharmacol, 7:78, (1961)	4000-12500	iu.l ⁻¹

APPENDIX C (Continued)

Urinalysis

Tests:	pH
	Specific Gravity
	Protein
	Glucose
	Ketones
	Bilirubin
	Blood
	Urobilinogen

All tests are performed using:	Multistix
	Ames Division
	Mill Laboratories Ltd
	Stoke Poges
	Slough SL2 2LY

Urine samples showing the presence of protein or blood will be spun and a sample of the spun deposit examined microscopically.

APPENDIX D

Metropolitan Life Insurance

Height and Weight Standards

Males

HEIGHT (cm) In shoes 2.5cm heels	WEIGHT IN INDOOR CLOTHING (kg)		
	SMALL FRAME	MEDIUM FRAME	LARGE FRAME
157.5	58.1-60.1	59.5-64.0	62.6-68.0
160.0	59.0-61.7	60.4-64.9	63.6-69.5
162.6	59.9-62.7	61.7-65.8	64.5-70.8
165.1	60.8-63.6	62.2-67.2	65.4-72.6
167.6	61.7-64.5	63.1-68.6	66.3-74.5
170.2	62.7-65.8	64.5-69.9	67.6-76.3
172.7	63.6-67.2	65.8-71.3	69.0-78.1
175.3	64.5-68.6	67.2-72.6	70.4-79.9
177.8	65.4-69.9	68.6-74.0	71.7-81.7
180.3	66.3-71.3	69.9-75.4	73.1-83.5
182.9	67.6-72.6	71.3-77.2	74.5-85.4
183.4	69.0-74.5	72.6-79.0	76.3-87.2
188.0	70.4-76.3	74.5-80.8	78.1-89.4
190.5	71.7-78.1	75.8-82.6	79.9-91.7
193.0	73.5-79.9	77.6-84.9	82.2-94.0

These are 1983 Metropolitan Life Insurance company weight tables by height and size of frame, for people aged 25 to 59, in shoes, wearing 2.3kg of indoor clothing for men, 1.4kg for women.

APPENDIX D (continued)

Metropolitan Life Insurance

Height and Weight Standards

Females

HEIGHT (CM)	WEIGHT IN INDOOR CLOTHING (Kg)		
In shoes 5cm heels	SMALL FRAME	MEDIUM FRAME	LARGE FRAME
147.3	46.3-50.4	49.5-54.9	53.6-59.5
149.9	46.7-51.3	50.4-61.3	54.5-80.8
152.4	47.2-52.2	51.3-57.2	55.4-62.2
154.9	48.1-53.6	52.2-58.6	56.8-63.6
157.5	49.0-54.9	53.6-59.9	58.1-64.9
160.0	50.4-56.3	54.9-61.3	59.5-66.7
162.6	51.6-57.7	56.3-62.7	60.8-68.6
165.1	53.1-59.0	57.7-64.0	62.2-70.4
167.6	54.5-60.4	59.0-65.4	63.6-72.2
170.2	55.8-61.7	60.4-66.7	64.9-74.0
172.7	57.3-63.1	61.7-68.0	66.3-75.8
175.3	58.6-64.5	63.1-69.5	67.6-77.2
177.8	59.9-65.8	64.5-70.8	69.0-78.5
180.3	61.3-67.2	65.8-72.2	70.4-79.9
182.9	62.7-68.6	67.2-73.5	71.7-81.3

APPENDIX E**TOXICITY RATING SCALE**

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
HAEMATOLOGY				
Haemoglobin	9.5-10.5 g.dl ⁻¹	8.0-9.4 g.dl ⁻¹	6.5-7.9 g.dl ⁻¹	<6.5 g.dl ⁻¹
Absolute Neutrophil Count	1.00-1.50 x 10 ⁹ l ⁻¹	0.70-0.99 x 10 ⁹ l ⁻¹	0.50-0.69 x 10 ⁹ l ⁻¹	<0.50 x 10 ⁹ l ⁻¹
Platelet Count	75-99 x 10 ⁹ l ⁻¹	50-74 x 10 ⁹ l ⁻¹	20-49 x 10 ⁹ l ⁻¹	<20 x 10 ⁹ l ⁻¹
CLINICAL CHEMISTRY				
Hyponatraemia	130-134 mmol.l ⁻¹	123-129 mmol.l ⁻¹	116-122 mmol.l ⁻¹	<116 mmol.l ⁻¹
Hypernatraemia	150-154 mmol.l ⁻¹	155-158 mmol.l ⁻¹	159-165 mmol.l ⁻¹	>165 mmol.l ⁻¹
Hypokalaemia	3.0-3.3 mmol.l ⁻¹	2.5-2.9 mmol.l ⁻¹	2.0-2.4 mmol.l ⁻¹	<2.0 mmol.l ⁻¹
Hyperkalaemia	5.6-6.0 mmol.l ⁻¹	6.1-6.5 mmol.l ⁻¹	6.6-7.0 mmol.l ⁻¹	>7.0 mmol.l ⁻¹
Hypoglycaemia	3.1-3.6 mmol.l ⁻¹	2.2-3.0 mmol.l ⁻¹	1.7-2.1 mmol.l ⁻¹	<1.7 mmol.l ⁻¹
Hyperglycaemia *	6.5-8.9 mmol.l ⁻¹	9.0-13.9 mmol.l ⁻¹	14.0-28.0 mmol.l ⁻¹	>28.0 mmol.l ⁻¹

* Hyperglycaemia: non-fasting and no prior diabetes.

TOXICITY RATING SCALE (cont.)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
Hyperbilirubinemia	25.0-31.5 mmol.l ⁻¹	31.6-52.5 mmol.l ⁻¹	52.6-105.0 mmol.l ⁻¹	>105.0 mmol.l ⁻¹
Urea (18-40y) (40+)	10.4-20.8 mmol.l ⁻¹ 11.5-23.0 mmol.l ⁻¹	20.9-41.5 mmol.l ⁻¹ 23.1-46.0 mmol.l ⁻¹	41.6-83.0 mmol.l ⁻¹ 46.1-92.0 mmol.l ⁻¹	>83.0 mmol.l ⁻¹ >92.0 mmol.l ⁻¹
Creatinine (18-40y) (40+)	134-183 µmol.l ⁻¹ 150-204 µmol.l ⁻¹	184-366 µmol.l ⁻¹ 205-408 µmol.l ⁻¹	367-732 µmol.l ⁻¹ 409-816 µmol.l ⁻¹	>732 µmol.l ⁻¹ >816 µmol.l ⁻¹
AST	54-107 iu.l ⁻¹	108-215 iu.l ⁻¹	216-430 iu.l ⁻¹	>430 iu.l ⁻¹
ALT	56-112 iu.l ⁻¹	113-225 iu.l ⁻¹	226-450 iu.l ⁻¹	>450 iu.l ⁻¹
GGT	75-150 iu.l ⁻¹	151-300 iu.l ⁻¹	301-600 iu.l ⁻¹	>600 iu.l ⁻¹
Alkaline Phosphatase	349-697 iu.l ⁻¹	698-1395 iu.l ⁻¹	1396-2790 iu.l ⁻¹	>2790 iu.l ⁻¹
URINALYSIS				
Proteinuria	1+ or <0.3% or <3 g.l ⁻¹ or 200 mg - 1 gm loss.day ⁻¹	2-3+ or 0.3-1.0% or 3-10 g.l ⁻¹ or 1-2 gm loss.day ⁻¹	4+ or >1.0% or >10 g.l ⁻¹ or >2-3.5 gm loss.day ⁻¹	Nephrotic syndrome or >3.5 gm loss.day ⁻¹
Gross Haematuria	microscopic only	gross, no clots	gross + clots	obstructive or requires transfusion

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
RESPIRATORY				
Cough - for aerosol studies	transient - no Rx	treatment associated cough; local Rx	uncontrolled	
Bronchospasm acute	transient; no Rx, FEV1 <80%-70% (or peak flow)	req. Rx; normalises with bronchodilator; FEV1 50%-70% (or peak flow)	no normalisation w/bronchodilator; FEV1 25%-50% (or peak flow), retractions	cyanosis; FEV1 <25% (or peak flow) or intubated
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring therapy
NEURO/NEUROMUSCULAR				
Neuro-cerebellar	slight incoordination OR dysdiadochokinesia	intention tremor OR dysmetria OR slurred speech; nystagmus	locomotor ataxia	incapacitated
Neuro-psych/mood			severe mood changes requiring medical intervention	Acute psychosis requiring hospitalisation
Paraesthesia (burning, tingling etc.)	mild discomfort; no Rx required	mod discomfort; non-narcotic analgesia required	severe discomfort; OR narcotic analgesia required with symptomatic improvement	incapacitating; OR not responsive to narcotic analgesia

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
Neuro-motor	mild weakness in muscle of feet but able to walk and/or mild increase or decrease in reflexes	mod weakness in feet (unable to walk on heels and/or toes), mild weakness in hands, still able to do most hand tasks and/or loss of previously present reflex or development of hyperreflexia and/or unable to do deep knee bends due to weakness	marked distal weakness (unable to dorsiflex toes or foot drop) and mod proximal weakness eg in hands interfering with ADLs and/or requiring assistance to walk and/or unable to rise from chair unassisted	Confined to bed or wheel chair because of muscle weakness
Neuro-sensory	mild impairment (dec sensation eg vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution	mod impairment (mod dec sensation eg vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	Severe impairment (dec or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (ie upper and lower extremities)	Sensory loss involves limbs and trunk
Mood	mild anxiety or depression	therapy required for mod. depression OR mod. anxiety	needs assistance due to severe depression OR mania OR anxiety	acute psychosis or incapacitated or requires hospitalisation
Neuro-control (ADL = activities of daily living)	no Rx. req., ADLs unaffected AND mild agitation or diff. concentrating or confusion	min Rx., some ADL limitation AND mod. confusion or agitation	Rx. req., needs ADL assistance AND severe agitation or confusion	toxic psychosis or hospitalisation
Muscle strength	subjective weakness no objective symptoms/signs	mild objective signs, symptoms, no dec. in function	objective weakness; function limited	paralysis

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
CARDIAC				
Cardiac Arrhythmia		Asymptomatic; transient dysrhythmia, no Rx required	Recurrent/persistent dysrhythmia; symptomatic Rx required	Unstable dysrhythmia, hospitalisation and Rx required
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; no Rx required	requires treatment
Hypertension	transient inc. >20 mm/Hg no Rx	recurrent; chronic increase > 20 mm/Hg Rx req.	acute Rx required; outpatient hosp. possible	requires hospitalisation
Hypotension	transient orthostatic hypotension; no Rx	symptoms correctable with oral fluid Rx	requires IV fluids no hosp. required	requires hospitalisation
Pericarditis	minimal effusion	mild/mod asymp. effusion, no Rx	symptomatic effusion, pain, ECG changes	tamponade OR pericardiocentesis OR surgery required
Haemorrhage, blood loss		mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss, >2 units transfused
GASTROINTESTINAL				
Stomatitis	mild discomfort, no limits on activity	some limits on eating/talking	eating/talking very limited	req. IV fluids
Nausea	mild OR transient; reasonable intake maintained	mod. discomfort; OR intake decreased for <3 days	severe discomfort OR minimal intake for >3 days	hospitalisation required
Vomiting	mild OR transient; 2-3 episodes per day OR mild vomiting lasting <1 week	mod OR persistent; 4-5 episodes per day; OR vomiting lasting >1 week	severe vomiting of all food/fluids in 24 hours OR orthostatic hypotension OR IV Rx required	hypotensive shock OR hospitalisation required

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
Oral Discomfort/ Dysphagia	mild discomfort, no difficulty swallowing	difficulty swallowing but able to eat and drink	unable to swallow solids	unable to drink fluids; IV fluids required
Constipation	mild	moderate	severe	distension with vomiting
Diarrhoea	mild OR transient; 3-4 loose stools per day OR mild diarrhoea lasting <1 week	mod OR persistent; 5-7 loose stools per day OR diarrhoea lasting >1 week	bloody diarrhoea; OR orthostatic hypotension OR >7 loose stools/day OR IV Rx required	hypotensive shock or hospitalisation required
OTHER PARAMETERS				
Fever; oral, >12 hours	37.7-38.5C or 100.0-101.5F	38.6-39.5C or 101.6-102.9F	39.6-40.5C or 103-105F	>40.5C or >105F
Headache	mild, no Rx required	mod or non-narcotic analgesia Rx	severe; OR responds to initial narcotic Rx	intractable; OR requiring repeated narcotic Rx
Fatigue	normal activity reduced <25%	normal activity reduced 25-50%	normal activity reduced >50%; can't work	unable to care for self
Allergic reaction	pruritus w/o rash	localised urticaria	generalised urticaria or angioedema	anaphylaxis
Cutaneous/Rash/ Dermatitis	erythema, pruritus	diffuse, maculopapular rash OR dry desquamation	vesiculation OR moist desquamation OR ulceration	ANY ONE: Mucous membrane involvement, suspected Stevens-Johnson (TEN), erythema multiforme, necrosis req surgery, exfoliative dermatitis

APPENDIX E (Continued)

ITEM	GRADE 1 SEVERITY	GRADE 2 SEVERITY	GRADE 3 SEVERITY	GRADE 4 SEVERITY
GUIDELINES FOR ESTIMATING GRADE OF SEVERITY FOR CONDITIONS NOT LISTED IN TABLE				
Criteria				
Karnofsky score or Self care ability, impact on activities of daily living (ADL) or Medical care needed	80-90 Transient or mild discomfort; no limitation on activity No therapy; monitor condition	60-70 Mild to moderate impact on activity; may be able to work full-time; some assistance may be needed May require minimal intervention and monitoring	40-50 Marked impact; ADLs limited; may work part- time with some assistance Requires medical care and possible hospitalisation	<40 Completely disabled, needs full assist with ADLs, unable to work Requires active medical intervention; hospitalisation or hospice care



Inveresk Research

TRANENT EH33 2NE SCOTLAND
TELEPHONE: +44 (0) 1875 614545

ICR STUDY NO: 013177

**A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.**

PROTOCOL STATUS: AMENDMENT 2 – 20 JANUARY 1999

CONFIDENTIAL		DATE
Compiled by	<i>JS</i>	26 Jan 99
Approved by	<i>ST</i>	27 Jan 99
Copied by	<i>L. BEN</i>	28 Jan 99
Number of Copies Made	10	28 Jan 99
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8. <i>Bioanalytical</i>	✓	
9. <i>Pharmacy (OL)</i>	✓	
10. <i>ETHICS Committee (SIA)</i>	✓	
11. <i>Change/Study file</i>		



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REGISTERED IN SCOTLAND NUMBER 10987

ICR 013177 – AMENDMENT 2 – 20 JANUARY 1999

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Dr N Watson

Dr S J Mair

Dr J Dickson

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ICR 013177 – AMENDMENT 2 – 20 JANUARY 1999

Sponsor Contact Details

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Persons authorised to sign protocol and amendments

Sponsor: Dr C F Wilkinson
ICR: Dr S Freestone
Dr J Dickson

Analysis of Urine Samples

Dr Linda Aston
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CA91367

USA

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SIGNATURE PAGE

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.

THE UNDERSIGNED AGREE TO PERFORM THE STUDY ACCORDING TO THIS
PROTOCOL.

Study Director: S. J. J. J. Date: 27 Jan 1999

Clinical Investigator: S. J. J. J. Date: 27 Jan 1999

Sponsor Representative: Chris F. Williams Date: January 28, 1999

ICR 013177 – AMENDMENT 2 – 20 JANUARY 1999

This amendment has been issued for the following reasons:-

- 1. Add Diane Gillies as Clinical Research Associate (p2 of 16).**
- 2. To add the dose level at which the females will be dosed (Section 4.1.1).**
- 3. To incorporate where the urine pharmacokinetic samples will be analysed in Section 4.7.3.**
- 4. Amendment to 'Quality Assurance' Section 10.**
- 5. Amendment to volunteer information to incorporate the dose level for the female group.**

4.1.1. Design of study Final Paragraph

It has been decided to dose the female group at the dose level 15.0mg.kg⁻¹ in the final session.

4.7.1 Collection and Analysis of Urine Samples

Study samples will be received by Pacific Toxicology Laboratories (PL) at 8160 Variel Avenue, Woodland Hills, CA 91367. The samples should consist of frozen urine in plastic tubes. The samples should arrive stored frozen on dry ice.

Upon arrival the samples shall be accessioned according to PTL SOP 1-SFM-910515-2. At the time of accessioning, the condition of the samples shall be noted.

Study samples will be stored in a locked freezer maintained at -15°C. The temperature of the freezer will be recorded daily. The accessioner in charge of the locked freezer will record the accession numbers of the samples being placed in the freezer and the date of their arrival on the Sample Storage Record Sheet in the Sample Storage Log.

Upon commencement of analysis, the samples listed on the load list will be retrieved from the freezer. The date and time of the removal of the samples from the freezer will be recorded on the Sample Storage Record Sheet. The technician receiving the samples will initial the Sample Storage Record Sheet.

The technician will bring the samples to room temperature. After thoroughly mixing the sample, an appropriate volume aliquot will be removed. The remainder of the samples will then be recapped and returned to the accessioner in charge of the locked freezer who will enter the time and date the samples are returned on the Storage Sample Record Sheet in the Sample Storage Log. The technician returning the samples will initial the Sample Record Sheet.

Samples will be discarded upon written notification from the study director.

10. QUALITY ASSURANCE

Quality Assurance inspections will be carried out during the clinical and reporting phases of the study. Phases selected for inspection may include (but will not be limited to):

- Dose Preparation
- Dosing and protocol compliance
- CRF data review
- Special Assay inspection

Phases selected for audit may include (but will not be limited to):

- Data Listings and tables
- Draft report
- Final report

Quality Assurance inspections will be carried out on critical phases in the execution of the study. Further inspections on routine, repetitive processes are also performed, although not necessarily on elements from this study.

These inspections and audits will be carried out according to the relevant Standard Operating Procedures by Quality Assurance personnel independent of the staff involved in the study. Records of these inspections and audits will be documented and distributed to management for review.

Appendix A

Volunteer Information p14 of 16 paragraph 1 has been amended to read

"There has been no significant inhibition of blood cholinesterase in men. A group of women will receive the same dose as the last group of men (15.0mg.kg⁻¹). This will be given to of 10 women (7 to receive active compound and 3 placebo)."

APPENDIX A

Volunteer Consent Form

This agreement is between the volunteer _____ and Inveresk Clinical Research Limited (hereinafter referred to as ICR), and provides for the volunteer to take part in experiments, trial and/or tests of a chemical compound or compounds.

FOR ALL STUDIES

I, the undersigned voluntarily agree to take part in

Protocol No: 013177

Descriptive Study Title: *A randomised double blind ascending single oral dose study with malathion to determine the no effect level on plasma and RBC cholinesterase activity.*

I understand that the investigation will involve the administration of

Name(s) of compounds: Malathion

being the compound under test.

- 2) *I have been given a full explanation by Dr of the nature, purpose and likely duration of the study and what I will be expected to do and I have been advised about any discomfort and possible ill-effects on my health or well-being which he/she believes may result. The information document given to me is attached (Appendix A pages 50 of 70 to 53 of 70.*
- 3) I have been given the opportunity to question Dr _____ on aspects of the study and have understood the advice and information given as a result.
- 4) I agree to Dr Freestone contacting my general practitioner [and teaching or university authority if appropriate] to make known my participation in the study and I authorise my general practitioner to report details of my relevant medical or drug history, in confidence.

APPENDIX A (Continued)

- 5) I agree to comply with any instruction given during the study and to cooperate faithfully with Dr Freestone and to tell him immediately if I suffer from any deterioration of any kind in my health or well-being or any unexpected or unusual symptoms however they may have arisen.
- 6) I agree that I will not seek to restrict the use to which the results of the study may be put and, in particular, I accept that they may be disclosed to regulatory authorities for compounds in the UK and elsewhere.
- 7) I understand that I am free to withdraw from the study at any time without needing to justify my decision.
- 8) The supervising doctor confirms that subject to overriding requirement of law necessitating the disclosure of documents relating to the study, the volunteer will not be referred to by name in any document concerning the study disclosed to any person not under the direct control of the supervising doctor;
- 9) ICR confirms that:
 - (i) I shall receive in consideration for completing the study, the sum of £450 from the supervising doctor and that I shall receive the sum in full if it is necessary for me to withdraw from the study for medical reasons associated with participation in it. If I withdraw from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if I withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising doctor;

APPENDIX A (Continued)

- (ii) In the event of my suffering any bodily injury caused directly by my participation in the study, I may elect to proceed in accordance with the following optional procedure.
- (a) Compensation will be paid to me by ICR without my having to prove that the injury arose through negligence or that the study compound was defective as set forth in the Association of the British Pharmaceutical Industry "Guidelines for medical experiments in non-patient human volunteers".
- (b) The amount of such compensation shall be calculated by reference to the amount of damages commonly awarded for similar injuries by an English court if liability is admitted, providing that such compensation may be reduced to the extent that I, by reason of contributory fault through my actions or my failure to act, am partly responsible for the injury.
- (c) Any dispute or agreement as to the application of clause 9(ii) (a) and (b) may at my option be referred to an arbitrator to be agreed between myself and ICR, or in the absence of agreement, to be appointed by the President of the Royal College of Physicians of London with power in the arbitrator to consult a barrister of 10 years standing in respect of any issue of law including the amount of damages to be awarded as payment of compensation;
- (d) The agreement shall be construed in accordance with English law and subject to clause 9(ii) (a), (b) and (c) above the English courts shall have sole jurisdiction over any dispute which may arise out of it.

APPENDIX A (Continued)

Signed by the volunteer :

Dated :

Signed for on behalf
of ICR by its duly
authorised
representative :

Dated :

I confirm that I
have explained the
nature, purpose and
possible hazards of
the above trial to :

Signed :

Dated :

I confirm that I have witnessed the above explanation :

Signed :
Witness Signature

Dated :

(NB-It may be appropriate for the supervising doctor to fulfil the obligations of the
duly authorised representative for the company.

Since signing the consent, the volunteer information sheet has been changed. I confirm
that I have read the revised information (dated / /) and still consent to participation in the
study.

Signed :

Dated :

APPENDIX A (Continued)

Volunteer information

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND RBC CHOLINESTERASE ACTIVITY.

Introduction

Malathion is an organophosphorus (OP) insecticide, one of a family of cholinesterase inhibitor compounds that includes many widely used insecticides. These pesticides have essentially no effects on mammals at sufficiently low levels. At higher levels they reduce the amount of an enzyme (cholinesterase) that breaks down a chemical substance (acetylcholine) in the body that is responsible for the transmission of nerve impulses, with the effect increasing as the dose increases. At yet higher levels, sufficient to reduce considerably the levels of acetylcholine in the nervous system, mild to serious physical effects can result (see the paragraph entitled Side Effects, below). Of all the OP insecticides, malathion has been shown to be among the lowest in acute toxicity (toxicity from a single dose) in animals and humans.

Very small amounts of residues of malathion may exist at harvest on treated crops. To determine whether these residues pose any risk to food consumers, many toxicity studies in animals, and one human study, have already been conducted. The animal studies showed that single doses at levels well above those that will be involved in this study produced no reduction in cholinesterase levels and no toxic effects.

Although the mechanism by which OPs act to inhibit cholinesterase is well understood, differences in behaviour of compounds from species to species do exist. This study is being conducted to reduce the uncertainties of species differences in determining a level of human exposure that causes measured reduction of blood cholinesterase levels. Prior testing has shown that a relatively large reduction of blood cholinesterase is required before any resulting clinical effects are observed. The results of this study are expected to be useful in showing that the use of malathion on crops does not pose health risks to food consumers, and also may be useful in evaluating whether workers who use malathion are thereby at risk.

Aim

The aim of the study is to determine the highest dose at which no significant reduction of blood cholinesterase occurs.

Dose

The doses that have been given are 0.5, 1.5, 5.0, 10.0 and 15.0 mg.kg⁻¹ body weight and placebo (inactive compound). There has been no significant inhibition of blood cholinesterase in men. A group of women will receive the same dose as the last group of men (15.0mg.kg⁻¹). This will be given to 10 women (7 to receive active compound and 3 placebo).

Allocation to receive active compound or placebo (inactive compound) will be randomised. The compound or placebo will be administered as capsules by mouth.

APPENDIX A (Continued)

Volunteer information

Side Effects

In view of the low doses of malathion to be used in this study and the results of earlier studies, it is not anticipated that any of the subjects of the study will experience any adverse effects other than a reduced level of blood cholinesterase. However, human studies with other more potent organophosphorus compounds suggest that the following effects can result from sufficiently high doses. Initial symptoms typically are headache and nausea, followed by a feeling of chest tightness and coughing. At dose levels much higher than those being used in this study other possible symptoms include vomiting, diarrhoea, abdominal pain, blurred vision, weakness, sweating, constricted pupils, excessive saliva preparation, slow pulse, and involuntary muscle twitching or spasm. A few reported cases of coma or death from ingestion of large quantities of malathion have been reported. However, the doses that cause these effects are very much higher than the ones to be used in this study. In some animal studies (but not all) it has been shown that lifetime exposure to 1600 mg/kg daily of malathion (*ie* more than 100 times the maximum dose in this study every day for a lifetime) in sensitive species caused an increased incidence of liver cancer. The dose level and duration of treatment bears no relationship to the single low doses to which volunteers will be exposed in this study.

Procedure

You will attend ICR for screening within 21 days of the start of the study. At screening, a complete medical history will be taken and you will have a complete physical examination, recordings of your pulse and blood pressure obtained, an ECG recorded (*tracing of your heart's electrical activity*) and blood samples taken for various safety tests. Samples of blood will also be taken to test for hepatitis B, hepatitis C and HIV, the virus that can cause AIDS. Urine will also be tested including a test for drugs of abuse.

Your GP will be informed of your participation and asked to confirm your medical history. If there are any objections expressed by your GP you will be excluded from the study.

Once you have successfully passed the screening examination, including acceptable blood and urine test results, your co-operation with the following will be required:

1. Up to a total of 48 of you will be studied.
2. If you smoke you must be able to abstain from smoking from 2h predose to 8h postdose.
3. You will require to attend the clinic for 4 outpatient visits on 9, 7, 5 and 2 days prior to dosing when a blood sample will be taken.
4. You will then be resident in the clinic on one occasion for 3 nights and will then return for 3 further outpatient visits 3, 6 and 13 days after dosing.

APPENDIX A (Continued)

Volunteer Information

5. You will be admitted to the clinic between 10am and mid-day on the morning preceding the day of dosing. A brief examination including vital signs will be performed, and a blood sample taken for measurement of cholinesterase. A urine pregnancy test will be undertaken on female subjects. You will take no food or drink from 2300h.
6. On the day of dosing, you will be given breakfast and 5 min after completion you will receive either the active compound or placebo with 150 ml of water in the sitting position. You will then be required to remain seated or recumbent until 8h after dosing. You will be allowed water, fruit juice or decaffeinated drinks from approximately 3h after dosing. A light lunch will be provided approximately 4h after dosing. Normal activities excluding strenuous exercise will be allowed from approximately 4h after dosing.
7. Before dosing, a cannula (plastic tube inserted by a needle into a vein) will be inserted into your arm in order to allow samples of your blood to be obtained at regular intervals throughout the day of dosing. A needle and syringe can be used repeatedly to obtain blood samples if preferred. Repeated blood tests and cannulae can cause soreness and bruising of the arms or even, rarely, blockage of a vein, but those problems usually clear up within a few days to a few weeks.
8. Blood pressure, heart rate and a tracing of your heart's electrical activity will be recorded at intervals before and after dosing.
9. You will also be monitored for the presence or absence of the clinical signs listed in the introduction. A continuous recording of your heart's electrical activity will be displayed on a bedside monitor from 30mins before dosing to 4h after dosing.
10. You will be discharged 48h after dosing. Before discharge, a physical examination will be performed and blood samples will be taken for safety assessments.
11. You will return to the clinic in the morning 3, 6 and 13 days after dosing when a blood sample will be taken and to ensure continued well-being and for completion of any outstanding enquiry/adverse events.
12. Approximately 235 ml of blood will be taken during the study (compared with 480ml which is a standard blood donation).
13. You should avoid medication (including over-the-counter products) for 5 days before the start of the study.
14. You will not be allowed to take alcohol or other drugs on each resident study day or until after the last blood sample has been taken. It is recommended that you should refrain from alcohol as far as possible until the follow-up visit on Day 14.

If anything abnormal occurs, judged by the supervising clinician, or if laboratory investigations change, you may be withdrawn from the study. In addition, you may withdraw at any time without needing to justify your decision. (You are strongly advised not to leave the clinical unit within 24h after dosing as this may involve risk to your

APPENDIX A (Continued)

Volunteer information

health). If you decide to withdraw before completion of the study a medical examination including blood pressure and heart rate, blood sampling, urine sampling and an ECG will be performed.

You should inform the supervising physician of any symptoms. After the study is over, you will be given a telephone number to call if you have any questions or worries.

It is essential that you should adhere to all of these requirements. The supervising physician will be pleased to supply any further information at any time.

All information will be treated in a confidential manner but anonymised data will be seen by authorised persons involved in the study and possibly by compound regulating authorities.

Supervising Physicians

Dr J Dickson and Dr S Freestone
Inveresk Clinical Research
Riccarton
Edinburgh
EH14 4AP Tel: (0131) 451 5080



Inveresk Research

TRANENT EH33 3NE SCOTLAND
TELEPHONE: +44 (0) 1875 614343

Jellinek, Schwartz & Connolly Incorporated
1525 Wilson Boulevard
Suite 600
Arlington
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USA

29 March 1999

PROTOCOL TITLE:

A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to
Determine the No Effect Level on Plasma and RBC Cholinesterase Activity

ICR STUDY NO: 013177
Amendment 3

TEST MATERIAL: Malathion

INVERESK PROTOCOL CODE: Final

STUDY DIRECTOR: S Freestone MBChB FRCPE MD

PRINCIPAL INVESTIGATOR: D L Scott HND LRSC
(Inveresk Research)

PRINCIPAL INVESTIGATOR: L Aston BA MA PhD
(Pacific Toxicology Laboratories)

AMENDMENT 3
APPROVED BY: S. J. Freestone DATE: 1 Apr 99
Study Director

AMENDMENT 3
ACCEPTED BY: [Signature] DATE: 7 Apr 99
Principal Investigator (GLP) - Inveresk Research

AMENDMENT 3
ACCEPTED BY: [Signature] DATE: [Signature]
Principal Investigator (GLP) - Pacific Toxicology Laboratories

AMENDMENT 3
ACCEPTED BY: C. F. Williams DATE: 4/7/99
on behalf of the Sponsor

(No. of pages: 10
excluding front page)



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REGISTERED IN SCOTLAND, NUMBER 0175

MALATHION

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY

ICR STUDY NO. 013177

AMENDMENT 3

1 REASONS FOR AMENDMENT

- 1.1 To detail the analysis of plasma and urine samples for the determination of malathion and its metabolites under GLP.
- 1.2 To incorporate the GLP statement into Section 4.7, and remove it from Section 9.
- 1.3 To incorporate the QA, reporting and archiving aspects of the Bioanalytical phases of the study into Section 4.7.
- 1.4 To amend the timeplan.

AMENDMENT 3

2 Section 4.7 is replaced as follows:

**4.7 Collection And Analysis Of Samples For The Determination Of
Malathion And Its Metabolites**

4.7.1 COLLECTION OF BLOOD SAMPLES

Samples will be collected for the measurement of malathion and its malaoxon metabolite concentrations at the following timepoints:

Predose (0), 1, 2, 4, 8, 12, 48 and 72 h postdose.

Blood (ca 14 ml) will be collected via a cannula or by repeated venepuncture into two 7 ml lithium heparin tubes. Plasma will be obtained by centrifugation. There will be 432 plasma samples for analysis. A minimum of 5 ml of plasma is required for analysis, therefore the plasma obtained from both blood tubes will be pooled as 1 plasma sample and stored at ca -70°C. Samples will be transferred frozen in dry ice to:

Mr D L Scott (Principal Investigator)
Department of Bioanalytical Chemistry
Inveresk Research
Tranent
EH33 2NE
SCOTLAND

4.7.2 ANALYSIS OF PLASMA SAMPLES

4.7.2.1 MATERIALS

Test Materials for use as Analytical Standards

The test materials to be used as analytical standards (malathion and malaoxon) will be supplied (in suitable containers and under appropriate conditions) by the Sponsor.

Prior to the commencement of the study, the Sponsor will supply completed Test Material Data Sheets (TMDS) indicating the test materials identity, purity, stability, appearance, handling and safety instructions. The TMDS may cross refer to the Sponsor's Certificates of Analysis.

As instructed by the Sponsor, these test materials will be stored in the dark at -10°C or below.

Archive samples of the test materials will be retained (under the same storage conditions above) in the study archive at Inveresk Research, if sufficient material is supplied.

The Sponsor will supply written instructions regarding disposal or return of unused test material on completion of the study.

The malathion will be of analytical grade, Batch No. 344-055-54C.
The malaoxon will be of analytical grade, Batch No. 279-ABB-09-01.

Internal Standard

If it is decided that an internal standard is needed, then it will be supplied (in suitable containers and under appropriate conditions) by the Sponsor together with appropriate analytical certification, handling procedures and storage conditions.

Other Materials

Other materials will be obtained by Inveresk Research. Chemicals will be of analytical grade where available.

4.7.2.2 LOCATION OF STUDY

The analysis of plasma samples for malathion and malaoxon will be conducted in the Department of Bioanalytical Chemistry, Inveresk Research, Tranent EH33 2NE, Scotland.

4.7.2.3 EXPERIMENTAL PROCEDURE

The plasma samples will be stored immediately on receipt in the Department of Bioanalytical Chemistry at ca -70°C until analysed, using methodology validated at Inveresk (Analytical Method No. 6674, Inveresk Project No. 366748, Inveresk Report No. 17123).

The study plasma samples will be extracted and analysed in batches along with calibration standard and quality control samples prepared at suitable concentrations as determined during the validation of Method No. 6674.

Each analytical batch will consist of:

A double blank sample (control human plasma with no malathion, malaoxon or internal standard added, if used) and a single blank sample (control human plasma with internal standard only added, if used) as applicable.

At least 5 calibration standards, different from zero, prepared the same day in control human plasma. The lowest calibration point for each analyte should correspond to the limit of quantification of the method for malathion and malaoxon respectively.

Quality control samples (in duplicate at least) will be prepared at the concentrations determined during the validation of Method No. 6674. These controls will be prepared the same day in control human plasma. The quality control samples will be distributed every 15 to 20 samples and at the end of the run.

A batch of samples will be accepted when the following criteria are met:

The back calculated value for each standard on the calibration curve will be within $\pm 15\%$ (and $\pm 20\%$ at the limit of quantification).

The accuracy and precision of the quality control samples are within $\pm 15\%$.

The concentration of malathion and malaoxon in each sample will be determined by weighted linear regression analysis and subsequent linear interpolation as determined during the validation of the analytical method.

4.7.2.4 STATISTICAL ANALYSIS OF RESULTS

Statistical analyses shall be limited to derivation of means, standard deviations, coefficients of variation and regression parameters as appropriate.

4.7.2.5 GOOD LABORATORY PRACTICE

This phase of the study will be conducted in accordance with the OECD Principles of Good Laboratory Practice as set forth by the United Kingdom Department of Health and as accepted by Regulatory Authorities throughout the European Community, United States of America (FDA and EPA) and Japan (MHW, MAFF and MITI).

All routine activities conducted during the course of this phase of the study are detailed in Inveresk Research's Standard Operating Procedures.

4.7.2.6 QUALITY ASSURANCE

Quality Assurance inspections will be carried out on critical phases in the execution of the Bioanalytical phase of the study. Further inspections on routine, repetitive processes are also performed, although not necessarily on materials from this study.

The Tables of Data, reporting the plasma results, will be audited.

These inspections and audits will be carried out by Quality Assurance personnel independent of staff involved in the study.

4.7.2.7 REPORTS

On completion of this phase of the study, Audited Tables of Data, reporting the study results (in ng.ml⁻¹ to 3 significant figures), will be issued to the Study Director for inclusion as an appendix to the clinical report.

4.7.2.8 ARCHIVE

Inveresk will retain in its archive, for a period of fifteen years (or for such shorter period as, in the opinion of Inveresk, the quality of the material affords evaluation) following the date of Issue of the Audited Tables of Data, the undemoted materials relating to this phase of the project:

- Protocol, protocol amendments and correspondence
- Test material receipts
- Reference samples of test materials; if sufficient material is supplied
- All original data generated
- Audited Tables of Data, reporting the study results

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5

At the end of five years, Inveresk will contact the Sponsor for instructions on the transfer, retention or disposal of materials. Fees for the transfer or continued retention of the material will be invoiced to the Sponsor. Disposal will be undertaken free of charge.

Biological samples generated during the course of this study will be held deep frozen for a period of 16 weeks following the date of issue of the Audited Tables of Data. These samples will then be disposed of unless Inveresk receives prior written instructions regarding shipment of the samples to the Sponsor or continued storage at Inveresk at the Sponsor's expense.

4.7.3 COLLECTION OF URINE SAMPLES

Urine will be collected for the measurement of malathion mono- and di-carboxylic acids and dimethylphosphate, dimethylthiophosphate and dimethyldithiophosphate at the following times:

Predose (-12 – 0 h), 0-12, 12-24 and 24-48 h postdose.

Urine volume will be measured and 2 x 20 ml aliquot from each period will be frozen at -70°C until analysis. There will be 192 urine samples for analysis. Samples will be transferred frozen in dry ice to:

Dr L Aston (Principal Investigator)
Pacific Toxicology Laboratories
6160 Variel Avenue
Woodlands Hills
CA 91367
USA

Upon arrival the samples shall be accessioned according to PTL SOP 1-SFM-91051502. At the time of accessioning, the condition of the samples shall be noted.

Study samples will be stored in a locked freezer maintained at -15°C. The temperature of the freezer will be recorded daily. The accessions in charge of the locked freezer will record the accession numbers of the samples being placed in the freezer and the date of their arrival on the Sample Storage Record Sheet in the Sample Storage Log.

Upon commencement of analysis, the samples listed on the load list will be retrieved from the freezer. The date and time of the removal of the samples from the freezer will be recorded on the Sample Storage Record Sheet. The technician receiving the samples will initial the Sample Storage Record Sheet.

The technician will bring the samples to room temperature. After thoroughly mixing the sample, an appropriate volume aliquot will be removed. The remainder of the samples will then be recapped and returned to the accessioner in charge of the locked freezer who will enter the time and date the samples are returned on the Storage Sample Record Sheet in the Sample Storage Log. The technician returning the sample will initial the Sample Storage Log. The technician returning the samples will initial the Sample Record Sheet.

Samples will be discarded upon written notification from the Study Director.

4.7.4 ANALYSIS OF URINE SAMPLES

4.7.4.1 MATERIALS

Test Materials for use as Analytical Standards

The test materials to be used as analytical standards (malathion mono- and di-carboxylic acids and dimethylphosphate, dimethylthiophosphate and dimethyldithiophosphate) will be supplied (in suitable containers and under appropriate conditions) by the Sponsor.

Prior to the commencement of the study, the Sponsor will supply a completed Test Material Data Sheets (TMDS) indicating the test materials identity, purity, stability, appearance, handling and safety instructions. The TMDS may cross refer to the Sponsor's Certificates of Analysis.

As instructed by the Sponsor, these test materials will be stored in the dark at -20°C or below.

Archive samples of the test materials will be retained (under the same storage conditions above) in the study archive at Pacific Toxicology Laboratories (PTL) if sufficient material is supplied.

The Sponsor will supply written instructions regarding disposal or return of unused test material on completion of the study.

The malathion mono-carboxylic acid will be of analytical grade, Batch No. 275-MJH-82-1. The malathion di-carboxylic acid will be of analytical grade, Batch No. 167-BSe-71C. The dimethylphosphate will be of analytical grade, Batch No. 302-OSJ-50B. The dimethylthiophosphate will be of analytical grade, Batch No. 267-OSJ-54B. The dimethyldithiophosphate will be of analytical grade, Batch No. 291-BSe-62A.

Internal Standard

The internal standard, for both the malathion carboxylic acids and the alkyl phosphates methods, has been obtained by PTL from Chem Service (Fenthion, Batch No. 50-45B).

Other Materials

Other materials will be obtained by PTL. Chemicals will be of analytical grade where available.

4.7.4.2 LOCATION OF STUDY

The analysis of urine samples for malathion mono- and di-carboxylic acids and dimethylphosphate, dimethylthiophosphate and dimethyl dithiophosphate will be conducted at Pacific Toxicology Laboratories (PTL), Woodlands Hills, CA 91367, USA.

4.7.4.3 EXPERIMENTAL PROCEDURE

The urine samples will be analysed using methodology validated at PTL according to SOP: 2-QAP-891122-2, Appendix A.

The study urine samples will be extracted and analysed in batches along with calibration standard and quality control samples prepared at concentrations determined during the validation of Method Nos. 3-MALA-90710-4 and 3-MOPPS-990115-1.

Each analytical batch will consist of:

A double blank sample (control human urine with no metabolites or internal standard added, if used) and a single blank sample (control human urine with internal standard only added, if used) as applicable.

At least 5 calibration standards, different from zero, prepared the same day in control human urine. The lowest calibration point for each analyte should correspond to the limit of quantification of the method.

Quality control samples (in duplicate at least) will be prepared at the concentrations determined during the validation of Method Nos. 3-MALA-90710-4 and 3-MOPPS-990115-1. These controls will be prepared the same day in control human urine. The quality control samples will be distributed every 15 to 20 samples and at the end of the run.

Two levels of spiked samples of each set of analytes will be prepared in blank urine and stored in the same freezer as will be used for the storage of the study samples. Upon commencement of sample analysis, three samples of each group of spiked samples and one blank will be extracted and analysed every other week for twenty weeks according to the approved methods. Extra storage stability study samples will be stored frozen and analysed periodically.

A batch of samples will be accepted when the following criteria are met:

The back calculated value for each standard on the calibration curve will be within $\pm 15\%$ (and $\pm 20\%$ at the limit of quantification).

The accuracy and precision of the quality control samples are within $\pm 15\%$.

The concentration of malathion mono- and di-carboxylic acids and dimethylphosphate, dimethylthiophosphate and dimethyldithiophosphate in each sample will be determined by weighted linear regression analysis and subsequent linear interpolation as determined during the validation of the analytical method.

4.7.4.4 STATISTICAL ANALYSIS OF RESULTS

Statistical analyses shall be limited to derivation of means, standard deviations, coefficients of variation and regression parameters as appropriate.

4.7.4.5 GOOD LABORATORY PRACTICE

This phase of the study will be conducted in accordance with Good Laboratory Practice as set forth by the United States of America EPA (40 CFR Part 160).

All routine activities conducted during the course of this phase of the study are detailed in PTL's Standard Operating Procedures.

4.7.4.6 QUALITY ASSURANCE

Quality Assurance inspections will be carried out on critical phases in the execution of the Bioanalytical phase of the study.

The report of the urine data will be audited.

These inspections and audits will be carried out by Quality Assurance personnel at Pacific Toxicology Laboratories independent of staff involved in the study.

4.7.4.7 REPORTS

On completion of this phase of the study a draft report appendix will be issued to the Study Director for inclusion in the clinical report. The report appendix will incorporate:

Methods used
Review/discussion of results obtained from sample analysis
Review/discussion of results obtained from storage stability study
Tables/Appendices of numerical data
Conclusions reached.

4.7.4.8 ARCHIVE

All raw data pertaining to this phase of the study will be sent to Inveresk Research. Inveresk Research will retain in its archive, for a period of fifteen years or for such shorter period as, in the opinion of Inveresk Research, the quality of the material affords evaluation) following the date of issue of the report appendix, the undemoted materials relating to the project:

Protocol, protocol amendments and correspondence
Test material receipts
All original data generated
A copy of the report appendix reporting the study results

At the end of five years, Inveresk Research will contact the Sponsor for instructions on the transfer, retention or disposal of materials. Fees for the transfer or continued retention of the materials will be invoiced to the Sponsor. Disposal will be undertaken free of charge.

Biological samples generated during the course of this study will be held deep frozen (at PTL) for a period of 16 weeks following the date of issue of the final report appendix. These samples will then be disposed of unless Inveresk receives prior written instructions regarding shipment of the samples to the Sponsor or continued storage at PTL at the Sponsor's expense.

- 3 Section 10 (of Amendment 1) has been re-numbered as Section 9 and reads as follows:

9. QUALITY ASSURANCE

Quality Assurance inspections will be carried out during the clinical and reporting phases of the study. Phases selected for inspection may include (but will not be limited to):

Dose preparation
Dosing and protocol compliance
CRF data review
Special assay inspection.

Phases selected for audit may include (but will not be limited to):

Data listings and tables
Draft report
Final report.

Quality Assurance inspections will be carried out on critical phases in the execution of the study. Further inspections on routine, repetitive processes are also performed, although not necessarily on elements from this study.

These inspections and audits will be carried out according to the relevant Standard Operating Procedures by Quality Assurance personnel independent of the staff involved in the study. Records of these inspections and audits will be documented and distributed to management for review.

- 4 Section 11 (of Amendment 1) has been re-numbered as Section 10 and reads as follows:

10 SPECIAL CONDITIONS

The study will proceed only after documented acceptance of the protocol by the Sponsor has been received by ICR.

- 5 Section 12 (of Amendment 1) has been re-numbered as Section 11 and reads as follows:

11 TIMEPLAN

Study start:	October 1998
Clinical phase completed:	March 1999
Issue of draft report:	TBC
Issue of final report:	TBC

Compiled by: D E Marshall
Date: March 1999

FILE NOTE

Date: 14 April 1999

Sponsor: Jellinek, Schwartz & Connolly Incorporated
1525 Wilson Boulevard
Suite 600
Arlington
VA 22209
USA

Project No: 013177

Project Title: A Randomised Double Blind Ascending Single Oral Dose Study
with Malathion to Determine the No Effect Level on Plasma and
RBC Cholinesterase Activity

To: Project File (Jellinek, Schwartz & Connolly Incorporated),
S Freestone (ICR), QA

From: D E Marshall

An error was made in the preparation of Protocol Amendment 3 to ICR Study No.
013177. The 24 h timepoint was omitted from Section 4.7.1. The number of samples
(432) is correct.

FILE NOTE
APPROVED BY: S. Freestone DATE: 16 Apr 1999
S Freestone
Study Director



Inveresk Research

TRANENT EH33 3NE SCOTLAND
TELEPHONE: +44 (0) 1875 614545

Jellinek, Schwartz & Connolly Incorporated
1525 Wilson Boulevard
Suite 600
Arlington
VA 22209
USA

06 September 1999

PROTOCOL TITLE:

A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to
Determine the No Effect Level on Plasma and RBC Cholinesterase Activity

ICR STUDY NO: 013177
Amendment 4

TEST MATERIAL: Malathion

INVERESK PROTOCOL CODE: Final

STUDY DIRECTOR: S Freestone MBChB FRCPE MD

PRINCIPAL INVESTIGATOR: D L Scott HND LRSC
(Inveresk Research)

AMENDMENT 4
APPROVED BY: S. Freestone DATE: 7 Sept 1999
Study Director

AMENDMENT 4
ACCEPTED BY: [Signature] DATE: 6 Sept 99
Principal Investigator (GLP) - Inveresk Research

AMENDMENT 4
ACCEPTED BY: C.F. Williams DATE: Sept. 13, 1999
on behalf of the Sponsor

(No. of pages: 4
excluding front page)



FAX: +44 (0) 1875 614555
E-MAIL: inveresk_research@jsggroup.com
INVERESK RESEARCH INTERNATIONAL LTD
1525 WILSON BOULEVARD, SUITE 600, ARLINGTON, VA 22209, USA

The Sponsor will supply written instructions regarding disposal or return of unused test material on completion of the study.

The malathion will be of analytical grade, Batch No. 324-05J-54C. The malaoxon will be of analytical grade, Batch No. 279-ABB-09-01.

Internal Standard

If it is decided that an internal standard is needed, then it will be supplied (in suitable containers and under appropriate conditions) by the Sponsor together with appropriate analytical certification, handling procedures and storage conditions.

Other Materials

Other materials will be obtained by Inveresk Research. Chemicals will be of analytical grade where available.

3 Section 4.7.2.3 is replaced as follows:

4.7.2.3 EXPERIMENTAL PROCEDURE

The plasma samples will be stored immediately on receipt in the Department of Bioanalytical Chemistry at ca -70°C until analysed, using methodology validated at Inveresk (Analytical Method No. 6674, Inveresk Project No. 366748, Inveresk Report No. 17123).

The study plasma samples will be extracted and analysed in batches along with calibration standard and quality control samples prepared at suitable concentrations as determined during the validation of Method No. 6674.

Jellinek, Schwartz and Connolly Incorporated have requested that initially only the plasma samples from the subjects dosed at 15.0 mg.kg⁻¹ will be analysed along with any samples from the subjects dosed with placebo in the same session. In each case, only the samples up to 12 h post dose will be analysed. If no quantifiable amounts of malathion or malaoxon are present in these samples no further sample analysis will be required.

Each analytical batch will consist of:

A double blank sample (control human plasma with no malathion, malaoxon or internal standard added, if used) and a single blank sample (control human plasma with internal standard only added, if used) as applicable.

At least 5 calibration standards, different from zero, prepared the same day in control human plasma. The lowest calibration point for each analyte should correspond to the limit of quantification of the method for malathion and malaoxon respectively.

Quality control samples (in duplicate at least) will be prepared at the concentrations determined during the validation of Method No. 6674. These controls will be prepared on the day of analysis in control human plasma. The quality control samples will be distributed every 15 to 20 samples and at the end of the run.

A batch of samples will be accepted when the following criteria are met:

The back calculated value for each standard on the calibration curve will be within $\pm 15\%$ of the actual concentration (and $\pm 20\%$ at the limit of quantification).

The accuracy and precision of the quality control samples are within $\pm 15\%$ of the actual concentration.

The concentration of malathion and malaoxon in each sample will be determined by weighted linear regression analysis and subsequent linear interpolation as determined during the validation of the analytical method.

- 4 The entire 4.7.3 and 4.7.4 of Amendment 3 are deleted, and are replaced as follows:

4.7.3 COLLECTION OF URINE SAMPLES

Urine will be collected for the measurement of malathion mono- and di-carboxylic acids and dimethylphosphate, dimethylthiophosphate and dimethyldithiophosphate at the following times:

Predose (-12 – 0 h), 0-12, 12-24 and 24-48 h postdose.

Urine volume will be measured and 2 x 20 ml aliquot from each period will be frozen at -70°C until analysis. There will be 192 urine samples for analysis. Samples will be transferred frozen in dry ice to:

Dr L Aston
Pacific Toxicology Laboratories
8160 Varial Avenue
Woodlands Hills
CA 91367
USA

Inveresk Research

Inveresk 013177 (Final)
Amendment 4

4

4.7.4 ANALYSIS OF URINE SAMPLES

4.7.4.1 Sample analysis will be carried out under a separate protocol, PTL 119801, agreed between Cheminova Agro A/S and Pacific Toxicology Laboratories.

4.7.4.2 A final report including Quality Assurance and Study Director GLP authentication statements will be submitted to Inveresk Research for inclusion, as an Appendix, in the Inveresk report.

Compiled by: D L Scott
Date: September 1999



Inveresk Research

TRANENT EH33 3NE SCOTLAND
TELEPHONE: +44 (0) 1875 614345

ICR STUDY NO: 013177

**A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.**

PROTOCOL STATUS: AMENDMENT 4 – 25 NOVEMBER 1999

CONFIDENTIAL		DATE
Compiled by	<i>Dr. J. R. Miller</i>	<i>01 Dec 99</i>
Approved by	<i>Dr. J. R. Miller</i>	<i>1 Dec 99</i>
Copied by	<i>Dr. J. R. Miller</i>	<i>3/12/99</i>
Number of Copies Made	<i>3</i>	
Copies Distributed as Follows:-		
1. <i>Principal Investigator</i>	<i>SFR</i>	<i>3/12/99</i>
2. <i>Study Doctor</i>	<i>SEB</i>	<i>13/12/99</i>
3. <i>CRA</i>	<i>Chi</i>	<i>3/12/99</i>
4. <i>Client</i>		<i>02 Dec 99</i>
5. <i>Ethics</i>	<i>SW</i>	<i>02 Dec 99</i>
6.		
7.		
8.		
9.		
10		
11.		



FAX: +44 (0) 1875 614555
E-MAIL: inveresk_research@sigagroup.com

INVERESK CLINICAL RESEARCH LIMITED
RESEARCH OFFICE: ELPHINSTONE RESEARCH CENTRE TRANENT EH33 3NE
REGISTERED IN SCOTLAND NUMBER 19992

ICR 013177 – AMENDMENT 4 – 25 NOVEMBER 1999

Clinical Investigators

Dr W S Nimmo

Dr S Freestone

Dr N Watson

Dr S J Mair

Dr J Dickson

Dr G Dennis

Study Director

Dr S Freestone

Project Physician

Dr J Dickson

Project CRA (Clinical Research Associate)

K Whalley and Diane Gillies

Pharmacist

D Lyall

Trial Site

Inveresk Clinical Research

Research Park

Edinburgh EH14 4AP

SCOTLAND

Quality Assurance

C Brown

Clinical Pathology

P Hudson

Statistics and Data Management

D Chalmers

Laboratory

Inveresk Research

Elphinstone Research Centre

Tranent

Edinburgh EH33 2NE

SCOTLAND

Tel: 01875 614545

Fax: 01875 614555

Tel: 01875 614545

Fax: 01875 614555

ICR 013177 – AMENDMENT 4 – 25 NOVEMBER 1999

Sponsor Contact Details

Dr C F Wilkinson
Jellinek, Schwartz & Connolly Inc
1525 Wilson Boulevard
Suite 600
Arlington
VA 22209
USA

Tel: 001 703 312 8518

Fax: 001 703 527 5477

Sponsor Medical Contact

Dr Anna-Mette Nielsen

Emergency Number

Tel: 001 703 312 8518.

Persons authorised to sign protocol and amendments

Sponsor: Dr C F Wilkinson
ICR: Dr S Freestone
Dr J Dickson

Analysis of Urine Samples

Dr Linda Aston
Pacific Toxicology
6160 Variel Avenue
Woodland Hills
CA91367
USA
Fax:- 001 818 598 3116
Tel:- 001 818 598 3110

SIGNATURE PAGE

A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY
WITH MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND
RBC CHOLINESTERASE ACTIVITY.

THE UNDERSIGNED AGREE TO PERFORM THE STUDY ACCORDING TO THIS
PROTOCOL.

Study Director: S. Freestone Date: 2 Dec 1999

Clinical Investigator: J. Dickson Date: 1 Dec 99

Sponsor Representative: Jane Allery Date: December 7, 1999

ICR 013177 – AMENDMENT 4 – 25 NOVEMBER 1999

This protocol amendment has been issued to document that, at the request of the sponsor, the urine analysis performed by Pacific Toxicology Laboratories will be reported in a separate report and will not be included in the ICR report.

This amendment also documents a change in the QA department. Colin Brown replaced Jane Wood as head of QA department in July 1999.



Inveresk Research

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FILENOTE NO. 2

ICR Study No. 013177

Sponsor: Cheminova

This file note has been issued to clarify a typographical error in amendment 4 dated 25 November 1999. This amendment should have been documented as amendment 5 as an amendment 4 had previously been issued dated 06 September 1999.

Signed: S. J. Iredale
Study Director

Date: 8 Dec 1999

Signed: P. Gillies
Clinical Research Associate

Date: 05 Dec 1999

L:\data\file note\013177



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E-MAIL: inveresk.research@inveresk.com
INVERESK CLINICAL RESEARCH LIMITED
REGISTERED OFFICE: ALPHATOWNE RESEARCH CENTRE TRANENT EH43 2NE
REGISTERED IN SCOTLAND NUMBER 101987

Volunteer Number

Volunteer's Initials

Panel Number

**A RANDOMISED DOUBLE BLIND ASCENDING SINGLE ORAL DOSE STUDY WITH
MALATHION TO DETERMINE THE NO EFFECT LEVEL ON PLASMA AND RBC
CHOLINESTERASE ACTIVITY.**

STUDY NO. 013177

Study Director: Dr S Freestone MD FRCPEdin

Project Nurse: F Robertson RGN

Project Doctor: Dr J Dickson MBChB

Project CRA: K Whalley RGN RSCN

Inveresk Clinical Research
Riccarton
Edinburgh

Tel: 01875 614545

Sponsor: Cheminova Agro A/S

Tel: 001 703 312 8518 (Jellinek, Schwartz & Connolly Inc)

Fax: 001 703 527 5477 (Jellinek, Schwartz & Connolly Inc)

Sponsor Contact: Dr C F Wilkinson (from Jellinek, Schwartz & Connolly Inc)

STUDY NO. 013177

Volunteer Number ☐☐☐

Volunteer's Initials ☐☐☐

INVESTIGATOR'S STATEMENT

I certify that:

- 1 I have carefully examined all entries on pages 1 through 32 of the Case Report Form for this subject and,
- 2 All information entered in to the Case Report Form by me or my associates for this subject is, to the best of my knowledge, correct.

Investigator signature

Date

(This should be signed by the principal investigator, normally the Study Director)

MONITORING STATEMENT

Pages 1 through 32 of the Case Report Form for this subject have been monitored and checked for completeness and accuracy.

Primary Monitor

Date

Secondary Monitor

Date

Client Monitor
(if applicable)

Date

STUDY NO. 013177

Volunteer Number

Volunteer's Initials

SELECTION CRITERIA

38 male and 10 female volunteers aged between 18-50 years will be included in the study. Healthy volunteers will be selected from a volunteer panel held by Inveresk Clinical Research Ltd. The volunteers will have been declared healthy following a satisfactory history, medical examination and routine haematological and biochemical tests. The medical examination and tests are to be carried out not more than 3 weeks before commencement of the study.

NOTES FOR RECORDING

The Investigator will maintain adequate and accurate Case Report Forms to record all observations and other data pertinent to the clinical investigation. These forms are to be completed in their entirety in a neat legible manner to ensure accurate interpretation of data. Black ink should be used to ensure clarity of reproduced copy of all Case Report Forms. Corrections should be crossed through with a single line and the amendment initialled and dated. A reason for the correction should also be given. Correction fluid will not be used.

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

DEMOGRAPHIC DATA AND BACKGROUND INFORMATION	
Date of Birth	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year
Age	<input type="text"/> <input type="text"/> years
Sex (tick one)	<input type="checkbox"/> Male <input type="checkbox"/> Female
Race (tick one)	<input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic <input type="checkbox"/> Asian Other (specify) _____

SMOKING HISTORY	
Does subject smoke?	
Yes <input type="checkbox"/> No <input type="checkbox"/> Previously <input type="checkbox"/> (tick one)	
If yes, specify:	number of cigarettes per day <input type="text"/> <input type="text"/>
	number of cigars per day <input type="text"/> <input type="text"/>
	ounces of tobacco per day <input type="text"/> <input type="text"/>
If previously, specify:	Date of stopping <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

ALCOHOL INTAKE	
Does subject drink alcohol?	
Yes <input type="checkbox"/> No <input type="checkbox"/> (tick one)	
If yes, specify:	units per week <input type="text"/> <input type="text"/>

Signature: _____

Date:

(to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

PHYSICAL EXAMINATION	
Height (cm)	<input type="text"/> <input type="text"/> <input type="text"/>
Weight (kg)	<input type="text"/> <input type="text"/> <input type="text"/>
Caliper Size (cm)	<input type="text"/> <input type="text"/>
Frame Size (please tick)	Small
	Medium
	Large

Signature: _____
Date:
(to be signed by the person who records the data)

STUDY NO. 013177

Date:
 Day Month Year

Volunteer Number
 Volunteer's Initials

VITAL SIGNS										
	Supine Systolic Blood Pressure (mmHg)	Supine Diastolic Blood Pressure (mmHg)	Supine Pulse Rate (bpm)	Oral Temperature (°C)	Respirations (rpm)	Erect Pulse Rate (bpm)	Initials	*Repeat value to be used for analysis (Y/N)	Initials*	
Screening	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			
1 st repeat if required	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			
2 nd repeat if required (Confirmation)	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			
3 rd repeat if required	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			

* To be assigned and signed by a doctor.

Signature:
 Date:
 (to be signed by the person who records the data)

STUDY NO. 013177

Date:

Day Month Year

Volunteer Number

Volunteer's Initials

ECG FINDINGS								
Ventricular Rate (bpm)	* Rhythm	PR interval(s)	QRS interval(s)	QT interval(s)	QT _c interval(s)	QRS Axis	1= Normal 2= Abnormal	Comments
<input type="text"/> <input type="text"/> <input type="text"/>		<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/>	

- * SR = Sinus rhythm SA = Sinus arrhythmia
SB = Sinus bradycardia ST = Sinus tachycardia

Transcribed data. Original in results folder.

Signature: _____
Date:
(to be signed by the person who transcribes the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

Has the subject taken any medication in the last month? Yes ☐ No ☐ (tick one)

MEDICATION DURING THE PAST 1 MONTH			
	CODE*		CODE*
Drug (generic name)			
Route			
Dose			
Units			
Frequency			
Duration of Therapy	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
Indication:	 		
Side Effects (tick one) If yes, nature of side effects	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	

(* To be completed by coders from Phase I)

Signature: _____
Date:
(to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

MEDICAL HISTORY

	Yes	No	Details:
1. Eyes, ears, nose and throat			
2. Respiratory			
3. Cardiovascular			
4. Gastrointestinal			
5. Genitourinary			
6. Musculoskeletal			
7. Neurological			
8. Endocrine/metabolic			
9. Haemopoietic/lymphatic			
10. Dermatological			
11. Psychological			
12. Other chronic system disease			<u>Specify:</u>
13. Surgical history			<u>Specify:</u>
14. Allergies			<u>Specify:</u>

The details recorded above have been reviewed .

Signature: _____
Date:
(to be signed by a doctor)

Signature: _____
Date:
(to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

PHYSICAL EXAMINATION

General Appearance

	Normal	Abnormal	Comments:
Skin			
Hands			
Lymph Nodes			

Respiratory System

	Normal	Abnormal	Comments:
Chest movement			
Trachea			
Percussion			
Breath sounds			

Cardiovascular System

	Normal	Abnormal	Comments:
Peripheral Pulses			
JVP			
Heart Sounds			
Murmurs			

Signature: _____
Date:

(to be signed by the Doctor who performs the examination)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

PHYSICAL EXAMINATION

Gastrointestinal System

	Normal	Abnormal	Comments:
Mouth & Lips			
Abdomen:	Absent	Present	
Scars			
Tenderness			
Spleen	Not Palpable	Palpable	
Liver			
Kidneys	Normal	Abnormal	

Central Nervous System

	Normal	Abnormal	Comments:
Pupils			
Ophthalmoscopy			
Cranial Nerves			
Power			
Sensation			
Reflexes			
Cerebellar function			

Signature: _____

Date:

(to be signed by the Doctor who performs the examination)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

URINALYSIS	
Glucose	<input type="text"/>
Bilirubin	<input type="text"/>
Ketones	<input type="text"/>
Specific Gravity	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Blood	<input type="text"/>
pH	<input type="text"/> <input type="text"/>
Protein	<input type="text"/>
Urobilinogen	<input type="text"/> <input type="text"/> <input type="text"/>
Nitrites (if not applicable to study enter N/A)	N/A
Leucocytes (if not applicable to study enter N/A)	N/A
Urinalysis Normal? (tick one)	Yes <input type="checkbox"/> No <input type="checkbox"/>
Microscopy required? (tick one)	Yes <input type="checkbox"/> No <input type="checkbox"/>

URINE MICROSCOPY (If required)	
RBC	<input type="text"/> <input type="text"/> <input type="text"/> No per HPF
WBC	<input type="text"/> <input type="text"/> <input type="text"/> No per HPF
Casts	<input type="text"/> <input type="text"/> <input type="text"/> No per HPF
Microscopy Normal? Yes <input type="checkbox"/> No <input type="checkbox"/>	
(tick one)	
Comments: _____	

Transcribed data. Original in results folder.

Signature: _____
Date:
(to be signed by the person who transcribes the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

SAMPLE COLLECTION		
Sample		Initials
Clinical Chemistry	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Haematology	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Virology	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Cholinesterase (4.5ml EDTA)	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Urine Toxillab	Yes <input type="checkbox"/> No <input type="checkbox"/>	

PREGNANCY TEST (tick one)			
Negative	Positive	Not Applicable	Initials

Transcribed data. Original in results folder.

Signature: _____

Date:

(to be signed by the person who transcribes the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

CRITERIA FOR INCLUSION	Yes	No	Initials*	Date*
(a) Males and females 18 to 50 years of age				
(b) No clinically important abnormal physical findings at the screening examination.				
(c) No clinically relevant abnormalities in the results of the laboratory evaluation including plasma and RBC cholinesterase.				
(d) Normal ECG				
(e) Normal arterial pressure (BP) and heart rate (HR). These will be measured after resting supine for 3 minutes. Normal BP is taken to be 100 to 150 mm Hg systolic and 50 to 90 mm Hg diastolic. Normal HR is taken to be 50 to 90 bpm. Erect HR will be measured after standing for one minute. Normal Erect HR is taken to be 50 to 100 bpm.				
(f) Body weight between 50 and 100 kg and within +/- 15% of ideal body weight				
(g) Able to communicate well with the investigators 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				

NOTE: If the answer to any of these questions is NO, the volunteer is not eligible for the study.

(* to be dated and initialed by the person who answers each individual question - not necessarily the same for each one)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

CRITERIA FOR EXCLUSION	Yes	No	Initials*	Date*
(a) Administration of any investigational drug in the period 0 to 3 months before entry to the study (0-4 months if the previous investigational drug was a new chemical entity).				
(b) A need for any medication during the period 0 to 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 drug				
(d) Presence or history of allergy requiring treatment				
(e) Donation or loss of greater than 400ml of blood in the period 0 to 12 weeks before entry to 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 are not taking adequate contraceptive precautions.				
(j) Females with a positive pregnancy test.				
(k) Smokers who cannot abstain from smoking from 2h predose to 8h post dose.				
(l) Any subject with a resting pulse of < 45 bpm, a systolic BP of <100 mm Hg or a PR interval on ECG of >210 ms.				
(m) Any subject who has had exposure to anti-cholinesterases (including home pest control products) within one month of dosing.				
(n) All agricultural workers or pest control applicators.				

NOTE: If the answer to any of these questions is YES, the volunteer is not eligible for the study.

(* to be dated and initialled by the person who answers each individual question - not necessarily the same for each one)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

PRESTUDY
BLOOD SAMPLING

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day - 9	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	▲ 4.5	

Date:
Day Month Year

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day - 7	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	▲ 4.5	

Date:
Day Month Year

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day - 5	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	▲ 4.5	

Date:
Day Month Year

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day - 2	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	▲ 4.5	

▲ 4.5ml cholinesterase sample

Note: Samples should be taken at the same time of day if possible.

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

ADMISSION ASSESSMENT

Time of Admission (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		
	No	Yes
Has subject taken any alcohol in the last 48 hours?	<input type="text"/>	<input type="text"/>
		If yes, specify: <input type="text"/> <input type="text"/>
Has subject been unwell since the last visit?	<input type="text"/>	<input type="text"/>
		If yes, please complete an adverse event page
Has subject taken any medication in the last 5 days?	<input type="text"/>	<input type="text"/>
		If yes, please complete the concomitant medication page

Signature: _____

Date:

(to be signed by the person who records the data)

STUDY NO. 013177

Date:
 Day Month Year

Volunteer Number:
 Volunteer's Initials:

ADMISSION ASSESSMENT (CONTINUED)

VITAL SIGNS									
	Supine Systolic Blood Pressure (mmHg)	Supine Diastolic Blood Pressure (mmHg)	Supine Pulse Rate (bpm)	Oral Temperature (°C)	Respirations (rpm)	Erect Pulse Rate (bpm)	Initials	*Repeat value to be used for analysis (Y/N)	Initials*
Admission	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>		
Repeat if required	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>		
Confirmation if required	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>		

* To be assigned and signed by a doctor.

Signature:
 Date:
 (to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

ADMISSION ASSESSMENT (CONTINUED)

BLOOD SAMPLING			
Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day - 1	<input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	▲ 4.5	

▲ 4.5 ml cholinesterase sample

PREGNANCY TEST (tick one)			
Negative	Positive	Not Applicable	Initials

Transcribed data. Original in results folder.

Signature: _____

Date:

(to be signed by the person who transcribes the data)

Does the subject still fulfil the entry criteria? ☐ Yes ☐ No

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

DOSING		Initials
Has volunteer fasted from 2300 h on the evening before until breakfast?	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Time of breakfast completion (24h clock)	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	
Formulation administered whilst sitting?	Yes <input type="checkbox"/> No <input type="checkbox"/>	
150 mls of water given with formulation?	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Time of Administration (24h clock)	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	
Administered by (Signature)		
Checked by (Signature)		
Has volunteer remained seated or recumbent for 8 h post dose?	Yes <input type="checkbox"/> No <input type="checkbox"/>	

Please attach dose label below.

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

BLOOD SAMPLING					
Time after dose admin	Target Time (24h clock)	Actual Time (24h clock)	Volume of Blood taken (ml)	Comments	Initials
-30 min Predose	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	4.5		
Predose (0)	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● ξ 22		
1h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 18.5		
2h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 18.5		
4h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 18.5		
8h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 18.5		
12h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 18.5		
Date: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year					
24h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	ξ 26.5		
Date: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year					
48h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 18.5		

▲ cholinesterase sample (4.5 ml)
pk sample (14 ml)
ξ clinical chemistry and haematology (8 ml)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

CHOLINESTERASE RESULTS

VALUE	TIMEPOINT								
	Day -0 **	Day -7 **	Day -5 **	Day -2 **	Day -1 **	-30min **	Mean Baseline Value	Initials ∇	
Plasma ChE	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>
RBC ChE	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>	<div><div></div><div></div><div></div></div>

** Transcribed data. Original in results folder

25% Inhibition (plasma)	Initials ∇	Initials ∇	25% Inhibition value (RBC)	Initials ∇	Initials ∇
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		

Timepoint	Is % Inhibition ≥ 25% (plasma)		Initials •	Is % Inhibition ≥ 25% (RBC)		Initials •
	Yes *	No		Yes *	No	
1h						
2h						
4h						
8h						
12h						
24h						
48h						

* Any Inhibition equal to or greater than 25% to be reported immediately to medical staff. ∇ To be signed by 2 staff calculating mean values/inhibition
• To be signed by 2 staff checking inhibition.

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

MEALS				
Meal	Start Time (24h Clock)	Finish Time (24h Clock)	Comments	Initials
Breakfast	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		
Lunch (+4h)	TARGET TIME (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	ACTUAL TIME (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		

Subjects should refrain from fluids for 3h following dosing.
Decaffeinated drinks, fruit juice (not grapefruit juice) and water only will be allowed.

STUDY NO. 013177

Date:

Day Month Year

Volunteer Number

Volunteer's Initials

VITAL SIGNS										
Time after dose	Target Time (24h clock)	Actual Time (24h clock)	Supine Systolic BP (mmHg)	Supine Diastolic BP (mmHg)	Supine Pulse Rate (bpm)	Oral Temperature (°C)	Respirations (rpm)	Erect Pulse Rate (bpm)	Initials*	
Pre-dose	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
2h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
4h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
8h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
Date: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>										
Day Month Year										
24h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	

N.B. Repeat values to be recorded on the following page

* To be initialed by person recording the data.

Volunteer's Initials

[illegible]

25

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

CONTINUOUS ECG MONITORING			Init's
Has subject started continuous ECG Monitoring?	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
Time of Monitoring Start	Target Time (24h clock) <input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	Actual Time (24h clock) <input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	
Time of Monitoring Finish	Target Time (24h clock) <input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	Actual Time (24h clock) <input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	

ECG monitoring from -30 min predose until 4 h post dose
If any abnormalities detected, record on adverse event page

STUDY NO. 013177

Date:

Day Month Year

Volunteer Number

Volunteer's Initials

ECG FINDINGS									
Time after dose	Ventricular Rate (bpm)	Rhythm	PR Interval(s)	QRS Interval(s)	QT Interval(s)	QTc Interval(s)	QRS Axis	1 = Normal 2 = Abnormal	Initials †
Predose -30 min	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
2h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
4h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
8h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Date: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>									
Day Month Year									
24h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

Time after dose	Ventricular Rate (bpm)	Rhythm	PR Interval(s)	QRS Interval(s)	QT Interval(s)	QTc Interval(s)	QRS Axis	1 = Normal 2 = Abnormal	Initials †	If repeat, value to be used for inclusion in analysis (Y/N)**	Initials **
Post dose -h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
-h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
-h	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		

Time after dose	Comment on ECG (if required)
<input type="text"/>	<input type="text"/>

Transcribed data. Original in results folder.
SA = Sinus arrhythmia
SR = Sinus rhythm
SB = Sinus bradycardia
† To be initiated by the person who transcribes the data.
* Additional readings (should contain target time)
** To be completed by a doctor.

STUDY NO. 013177

Date:
 Day Month Year

Volunteer Number
 Volunteer's Initials

PK URINE COLLECTION						
Timepoint	Start time of collection (24h clock)	Completion time of collection (24h clock)	Volume (ml)	Has 1 x 20 ml sample been retained?	Initi *	Initi *
-12-0h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>		
Date: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year						
0-12h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>		
12-24h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>		
Date: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year						
24-48h	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>		

* To be signed by 2 staff handling samples.

Note: 20 ml samples should be stored at -70°C

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

24h POSTDOSE

URINALYSIS	
Glucose	<input type="text"/>
Bilirubin	<input type="text"/>
Ketones	<input type="text"/>
Specific Gravity	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Blood	<input type="text"/>
pH	<input type="text"/> <input type="text"/>
Protein	<input type="text"/>
Urobilinogen	<input type="text"/> <input type="text"/> <input type="text"/>
Nitrites (if not applicable to study enter N/A)	N/A
Leucocytes (if not applicable to study enter N/A)	N/A
Urinalysis Normal? (tick one)	Yes <input type="checkbox"/> No <input type="checkbox"/>
Microscopy required? (tick one)	Yes <input type="checkbox"/> No <input type="checkbox"/>

URINE MICROSCOPY (if required)	
RBC	<input type="text"/> <input type="text"/> <input type="text"/> No per HPF
WBC	<input type="text"/> <input type="text"/> <input type="text"/> No per HPF
Casts	<input type="text"/> <input type="text"/> <input type="text"/> No per HPF
Microscopy Normal? Yes <input type="checkbox"/> No <input type="checkbox"/>	
(tick one)	
Comments: _____	

Transcribed data. Original in results folder.

Signature: _____
Date:
(to be signed by the person who transcribes the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

COMPLETION EXAMINATION
(APPROXIMATELY +48H)

Have all adverse events been resolved (or documented as unresolved) prior to discharge?	Yes <input type="checkbox"/>	No <input type="checkbox"/>	(tick one)
---	------------------------------	-----------------------------	------------

Signature: _____
Date:
(to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

**COMPLETION EXAMINATION
(APPROXIMATELY +48H)**

General Appearance

	Any significant change from screen? (tick one)	Comments if changed:
Skin	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Hands	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Lymph Nodes	Yes <input type="checkbox"/> No <input type="checkbox"/>	

Respiratory System

	Any significant change from screen? (tick one)	Comments if changed:
Chest movement	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Trachea	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Percussion	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Breath sounds	Yes <input type="checkbox"/> No <input type="checkbox"/>	

Cardiovascular System

	Any significant change from screen? (tick one)	Comments if changed:
Peripheral Pulses	Yes <input type="checkbox"/> No <input type="checkbox"/>	
JVP	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Heart Sounds	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Murmurs	Yes <input type="checkbox"/> No <input type="checkbox"/>	

Signature: _____
Date:

(to be signed by the Doctor who performs the examination)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

**COMPLETION EXAMINATION
(APPROXIMATELY +48H)**

Gastrointestinal System

	Any significant change from screen? (tick one)	Comments if changed:
Mouth and Lips	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Abdomen: Scars	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Tenderness	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Spleen	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Liver	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Kidneys	Yes <input type="checkbox"/> No <input type="checkbox"/>	

Central Nervous System

	Any significant change from screen? (tick one)	Comments if changed:
Pupils	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Ophthalmoscopy	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Cranial Nerves	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Power	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Sensation	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Reflexes	Yes <input type="checkbox"/> No <input type="checkbox"/>	
Cerebellar Function	Yes <input type="checkbox"/> No <input type="checkbox"/>	

Signature: _____

Date:

(to be signed by the Doctor who performs the examination)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

INVESTIGATOR'S STATEMENT

I certify that:

- 1 I have carefully examined all entries on pages 33 through 42 of the Case Report Form for this subject and,
- 2 All information entered in to the Case Report Form by me or my associates for this subject is, to the best of my knowledge, correct.

Investigator signature

Date

(This should be signed by the principal investigator, normally the Study Director)

MONITORING STATEMENT

Pages 33 through 42 of the Case Report Form for this subject have been monitored and checked for completeness and accuracy.

Primary Monitor

Date

Secondary Monitor

Date

Client Monitor
(if applicable)

Date

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

POST STUDY

DAY 4			
	No	Yes	
Has subject been unwell since the last visit?	<input type="text"/>	<input type="text"/>	If yes, please complete an adverse event page.
Has subject taken any medication since the last visit?	<input type="text"/>	<input type="text"/>	If yes, please complete the concomitant medication page.

Signature: _____

Date:

(to be signed by the person who records the data)

Date:
Day Month Year

DAY 7			
	No	Yes	
Has subject been unwell since the last visit?	<input type="text"/>	<input type="text"/>	If yes, please complete an adverse event page.
Has subject taken any medication since the last visit?	<input type="text"/>	<input type="text"/>	If yes, please complete the concomitant medication page.

Signature: _____

Date:

(to be signed by the person who records the data)

Date:
Day Month Year

DAY 14			
	No	Yes	
Has subject been unwell since the last visit?	<input type="text"/>	<input type="text"/>	If yes, please complete an adverse event page.
Has subject taken any medication since the last visit?	<input type="text"/>	<input type="text"/>	If yes, please complete the concomitant medication page.

Signature: _____

Date:

(to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

POST STUDY
BLOOD SAMPLING

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day 4 (i.e. approximately 72h postdose)	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	● 18.5	

Date:
Day Month Year

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day 7	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	4.5	

Date:
Day Month Year

Timepoint	Time of Collection (24h clock)	Volume of Blood Taken (ml)	Initials
Day 14	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	4.5	

- ▲ cholinesterase sample (4.5 ml)
● pk sample (14 ml)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

Any new adverse event experienced? Yes ☐ No ☐

ADVERSE EVENT - EVENT 1

	Initia	Date
Nature of Event		
Additional Comments		
Date of Onset <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Session or N/A <input type="checkbox"/>		
Time of onset (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		
Predose? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Ongoing at end of study? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Date of resolution <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Time of resolution (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		
* Reporting of Event <input type="checkbox"/> * Severity of Event <input type="checkbox"/>		
Was adverse event serious? (To be completed by a doctor) Yes <input type="checkbox"/> No <input type="checkbox"/>		
* Relationship to study drug If 1 or 2 please give underlying cause <input type="checkbox"/>		
Coded term for event		
Study drug discontinued Yes <input type="checkbox"/> No <input type="checkbox"/>		
Treatment required to resolve adverse event If yes, please complete concomitant medication page. Yes <input type="checkbox"/> No <input type="checkbox"/>		

- * 1 = Volunteered by Subject
- 2 = Elicited by Questioning
- 3 = Observed by Investigator

- * 1 = Grade 1 Severity
- 2 = Grade 2 Severity
- 3 = Grade 3 Severity
- 4 = Grade 4 Severity
- Refer to Protocol Appendix E

- * 1 = Not related
- 2 = Unlikely
- 3 = Possibly related
- 4 = Probably related
- 5 = Definitely
- (To be assigned by 2 doctors)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

Any new adverse event experienced? Yes ☐ No ☐

ADVERSE EVENT - EVENT 2

	Initia	Date
Nature of Event		
Additional Comments		
Date of Onset <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Session or N/A <input type="checkbox"/>		
Time of onset (24h clock) <input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>		
Predose? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Ongoing at end of study? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Date of resolution <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Time of resolution (24h clock) <input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>		
^a Reporting of Event <input type="checkbox"/> ^b Severity of Event <input type="checkbox"/>		
Was adverse event serious? (To be completed by a doctor) Yes <input type="checkbox"/> No <input type="checkbox"/>		
^c Relationship to study drug If 1 or 2 please give underlying cause <input type="checkbox"/>		
Coded term for event		
Study drug discontinued Yes <input type="checkbox"/> No <input type="checkbox"/>		
Treatment required to resolve adverse event If yes, please complete concomitant mediation page. Yes <input type="checkbox"/> No <input type="checkbox"/>		

^a 1 = Volunteered by Subject
2 = Elicited by Questioning
3 = Observed by Investigator

^b 1 = Grade 1 Severity
2 = Grade 2 Severity
3 = Grade 3 Severity
4 = Grade 4 Severity
Refer to Protocol
Appendix E

^c 1 = Not related
2 = Unlikely
3 = Possibly related
4 = Probably related
5 = Definitely
(To be assigned by 2 doctors)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

Any new adverse event experienced? Yes ☐ No ☐

ADVERSE EVENT - EVENT 3

	Inits	Date
Nature of Event		
Additional Comments		
Date of Onset <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Session or N/A <input type="checkbox"/>		
Time of onset (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		
Predose? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Ongoing at end of study? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Date of resolution <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Time of resolution (24h clock) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		
^a Reporting of Event <input type="checkbox"/> ^b Severity of Event <input type="checkbox"/>		
Was adverse event serious? (To be completed by a doctor) Yes <input type="checkbox"/> No <input type="checkbox"/>		
^c Relationship to study drug If 1 or 2 please give underlying cause <input type="checkbox"/>		
Coded term for event		
Study drug discontinued Yes <input type="checkbox"/> No <input type="checkbox"/>		
Treatment required to resolve adverse event If yes, please complete concomitant mediation page. Yes <input type="checkbox"/> No <input type="checkbox"/>		

- ^a 1 = Volunteered by Subject
2 = Elicited by Questioning
3 = Observed by Investigator

- ^b 1 = Grade 1 Severity
2 = Grade 2 Severity
3 = Grade 3 Severity
4 = Grade 4 Severity
Refer to Protocol
Appendix E

- ^c 1 = Not related
2 = Unlikely
3 = Possibly related
4 = Probably related
5 = Definitely
(To be assigned by 2 doctors)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number
Volunteer's Initials

Any new adverse event experienced? Yes ☐ No ☐

ADVERSE EVENT - EVENT 4

	Initia	Date
Nature of Event		
Additional Comments		
Date of Onset <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Session or N/A <input type="checkbox"/>		
Time of onset (24h clock) <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>		
Predose? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Ongoing at end of study? Yes <input type="checkbox"/> No <input type="checkbox"/>		
Date of resolution <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Day Month Year		
Time of resolution (24h clock) <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>		
* Reporting of Event <input type="checkbox"/> ^b Severity of Event <input type="checkbox"/>		
Was adverse event serious? (To be completed by a doctor) Yes <input type="checkbox"/> No <input type="checkbox"/>		
* Relationship to study drug <input type="checkbox"/> If 1 or 2 please give underlying cause		
Coded term for event		
Study drug discontinued Yes <input type="checkbox"/> No <input type="checkbox"/>		
Treatment required to resolve adverse event If yes, please complete concomitant medication page. Yes <input type="checkbox"/> No <input type="checkbox"/>		

- * 1 = Volunteered by Subject
- 2 = Elicited by Questioning
- 3 = Observed by Investigator

- ^b 1 = Grade 1 Severity
- 2 = Grade 2 Severity
- 3 = Grade 3 Severity
- 4 = Grade 4 Severity
- Refer to Protocol Appendix E

- ^c 1 = Not related
- 2 = Unlikely
- 3 = Possibly related
- 4 = Probably related
- 5 = Definitely
- (To be assigned by 2 doctors)

STUDY NO. 013177

Date:

Day Month Year

Volunteer Number

Volunteer's Initials

Any new or changes to medications since screening? Yes ☐ No ☐ (tick one)

MEDICATION CHANGES SINCE SCREENING			
	CODE*		CODE*
Drug (generic name)			
Route			
Dose			
Units			
Frequency			
Duration of Therapy	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
Indication:			
Side Effects (tick one) If yes, nature of side effects	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Was medication prescribed whilst in clinic?	<input type="checkbox"/> Yes <input type="checkbox"/> No		

(* To be completed by coders from Phase I).

If yes, medication prescription sheet should be placed in results folder.

• Any side effects to be recorded as adverse event

Signature: _____

Date:

(to be signed by the person who records the data)

STUDY NO. 013177

Date:

Day Month Year

Volunteer Number

Volunteer's Initials

Any new or changes to medications since screening? Yes ☐ No ☐ (tick one)

MEDICATION CHANGES SINCE SCREENING			
	CODE*		CODE*
Drug (generic name)			
Route			
Dose			
Units			
Frequency			
Duration of Therapy	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	To <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	From <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
Indication:			
Side Effects (tick one) If yes, nature of side effects	<input type="checkbox"/> Yes <input type="checkbox"/> No		<input type="checkbox"/> Yes <input type="checkbox"/> No
Was medication prescribed whilst in clinic?	<input type="checkbox"/> Yes <input type="checkbox"/> No		<input type="checkbox"/> Yes <input type="checkbox"/> No

(* To be completed by coders from Phase I).

If yes, medication prescription sheet should be placed in results folder.

• Any side effects to be recorded as adverse event

Signature: _____

Date:
(to be signed by the person who records the data)

STUDY NO. 013177

Date:
Day Month Year

Volunteer Number

Volunteer's Initials

VOLUNTEER COMPLETION WITHDRAWAL RECORD

Did volunteer complete study? Yes ☐ No ☐

If no, please complete volunteer early trial termination record.

Termination date
dd mm yy

Date of last dose
dd mm yy

Reason(s) for Premature Termination (Please tick)	Yes	No	If yes, please specify
Lost to follow up (1)	<input type="checkbox"/>	<input type="checkbox"/>	
Adverse reactions (2)	<input type="checkbox"/>	<input type="checkbox"/>	
Found not to meet inclusion criteria (3)	<input type="checkbox"/>	<input type="checkbox"/>	
Administration (4)	<input type="checkbox"/>	<input type="checkbox"/>	
Dosage/medication error or protocol violation (5)	<input type="checkbox"/>	<input type="checkbox"/>	
Intercurrent illness (6)	<input type="checkbox"/>	<input type="checkbox"/>	
Other (7)	<input type="checkbox"/>	<input type="checkbox"/>	
Death (8)	<input type="checkbox"/>	<input type="checkbox"/>	

Initiator(s) of Discontinuation (Please tick)	Yes	No
Volunteer (1)	<input type="checkbox"/>	<input type="checkbox"/>
Investigator (2)	<input type="checkbox"/>	<input type="checkbox"/>
Trial Monitor (3)	<input type="checkbox"/>	<input type="checkbox"/>
Other (4) Specify: _____	<input type="checkbox"/>	<input type="checkbox"/>

Signature: _____
Date: